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# NMR assignments and structural characterization of new thiourea and urea kynurenamine derivatives nitric oxide synthase inhibitors

**Mariem Chayah,[a] M. Dora Carrión,[a] Miguel A. Gallo,[a] Duane Choquesillo-Lazarte[b] and M. Encarnación Camacho[a]***

## Introduction

The bioactive molecule of nitric oxide (NO) is an important second messenger that participates in regulation of cardiovascular, nervous and immune systems.[1] NO synthases (NOS) catalyze the oxidation of the amino acid L-arginine to L-citrulline and NO with NADPH and molecular oxygen as cosubstrates.[2] In mammalians, three isoforms of NOS have been identified: neuronal (nNOS), endothelial and inducible NOS.[3] These isoenzymes are involved in important biological processes and are implicated in many pathological diseases. Thus, overproduction of NO by nNOS has been associated with neurodegenerative disorders in Alzheimer's, Parkinson's or Huntington's diseases,[4–6] and the inducible isoform seems to be responsible for the massive NO production in pathologies such as arthritis, colitis, tissue damage, cancer or several inflammatory states.[7–9] In this way, many authors have focused their interest in the development of NOS selective inhibitors.

In a recent paper, the synthesis and biological evaluation of a novel family of kynurenamine derivatives bearing a thiourea or urea fragment **31–45**, which were designed and evaluated as NOS inhibitor agents, have been described.[10]

Although the structures of these derivatives have been determined by means of standard spectroscopic techniques ($^1$H and $^{13}$C NMR and MS), a detailed NMR study has been performed in some of them, in order to unequivocally corroborate their structures.

The present study reports the unambiguous assignment of each signal in the $^1$H and $^{13}$C NMR spectra in compounds **31–45**, using one-dimensional and two-dimensional resonance techniques. The spectra of nitro derivatives **1–30**, the precursors in the synthetic pathway, are also included.

## Experimental

### NMR spectra

Nuclear magnetic resonance spectra were recorded on 300-MHz $^1$H NMR and 75-MHz $^{13}$C NMR Agilent Varian Direct Drive, 400-MHz $^1$H NMR and 100-MHz $^{13}$C NMR Agilent Varian Direct Drive and 600-MHz $^1$H NMR and 150-MHz $^{13}$C NMR Agilent Varian Direct Drive spectrometers at 298 K. The following parameters were used in DEPT experiments: pulse width (PW) (135°), 9.0 ms; recycle time, 1 s; 0.5 J (CH) = 4 ms; 65 536 data points acquired and transformed from 1024 scans; spectral width, 15 KHz; and line broadening, 1.3 Hz. Chemical shifts ($\delta$) are quoted in parts per million (ppm) and are referenced to the residual solvent peak: $CDCl_3$, $\delta = 7.26$ ppm ($^1$H), $\delta = 77.4$ ppm ($^{13}$C); $(CD_3)_2CO$, $\delta = 2.05$ ppm ($^1$H), $\delta = 29.84$ ppm ($^{13}$C); $(CD_3)_2SO$, $\delta = 2.50$ ppm ($^1$H), $\delta = 39.52$ ppm ($^{13}$C); and $CD_3OD$, $\delta = 3.31$ ppm ($^1$H), $\delta = 49.00$ ppm ($^{13}$C). Spin multiplicities are given as s (singlet), bs (broad singlet), d (doublet), dd (doublet doublet), ddd (doublet, doublet doublet), t (triplet), q (quadruplet) and m (multiplet). Coupling constant ($J$) are given in hertz.

The HMBC spectra were measured with a pulse sequence gc2hmbc (standard sequence, Agilent Vnmrj_3.2A software) optimized for 8 Hz (inter-pulse delay for the evolution of long-range couplings: 62.5 ms). The HSQC spectra were measured with a pulse sequence gc2hsqcse (standard sequence, Agilent Vnmrj_3.2A software).

Nuclear Overhauser spectra were recorded on an Agilent Varian Direct Drive spectrometer, operating at 600 MHz, with a spectral widths of 4.96 KHz in both F2 and F1 domains; 744 × 200 data points were acquired with 16 scans per increment and relaxation delays of 1.0 s. The mixing time in NOESY experiments was 0.5 s. Data processing was performed on a 1 × 1 K data matrix.

### Molecular dynamics simulations

Unrestricted molecular dynamics (MD) simulations were carried out with the AMBER 12 software suite[11] in explicit solvent using the AMBER force field leaprc ff12SB. Geometry of compound **39** was optimized using RHF/6-31G* as implemented in Gaussian 09 (Gaussian, Inc., Wallingford, CT). Charges were then assigned to

* *Correspondence to: M. Encarnación Camacho. Departamento de Química Farmacéutica y Orgánica, Facultad de Farmacia, Universidad de Granada, c/ Campus de Cartuja, s/n 18071 Granada, Spain. E-mail: ecamacho@ugr.es*

a *Departamento de Química Farmacéutica y Orgánica, Facultad de Farmacia, Universidad de Granada, Granada, Spain*

b *Laboratorio de Estudios Cristalográficos, IACT-CSIC, Armilla, Granada, Spain*

individual atoms by fitting the quantum mechanically calculated (RHF/6-31G*//RHF/3-21G*) molecular electrostatic potential to a point charge model. Compound **39** was immersed in a cubic box of $CHCl_3$ molecules (CL3 model) with a minimum distance of 12 Å from any atom to the edge. The particle mesh Ewald method for long-range electrostatic interactions together with standard periodic boundary conditions was used. SHAKE algorithm was applied to reach an integration step of 2.0 fs. The followed MD simulation protocol was the same as previously described.[10] Distances and conformational clustering were performed using the program cpptraj implemented in Amber Tools 14.

**Table 1.** Structural data of the intermediate (**1–30**) and final (**31–45**) compounds

| Compound | $R_1$ | $R_2$ | X | Compound | $R_1$ | $R_2$ | X |
|---|---|---|---|---|---|---|---|
| **1, 16, 31** | H | Me | S | **10, 25, 40** | H | Et | O |
| **2, 17, 32** | H | Et | S | **11, 26, 41** | H | Pr | O |
| **3, 18, 33** | H | Pr | S | **12, 27, 42** | OMe | Et | O |
| **4, 19, 34** | OMe | Me | S | **13, 28, 43** | OMe | Pr | O |
| **5, 20, 35** | OMe | Et | S | **14, 29, 44** | Cl | Et | O |
| **6, 21, 36** | OMe | Pr | S | **15, 30, 45** | Cl | Pr | O |
| **7, 22, 37** | Cl | Me | S | | | | |
| **8, 23, 38** | Cl | Et | S | | | | |
| **9, 24, 39** | Cl | Pr | S | | | | |

### X-ray crystallography

A yellow crystal of compound **39** was mounted on a MiTeGen Micromounts, and this sample was used for data collection. Data were collected with a Bruker D8 Venture diffractometer. Data were processed with APEX[12] and corrected for absorption using SADABS.[13] The structures were solved by direct methods,[14] which revealed the position of all non-hydrogen atoms. These atoms were refined on $F^2$ by a full-matrix least-squares procedure using anisotropic displacement parameters.[14] All hydrogen atoms were located in different Fourier maps and included as fixed contributions riding on attached atoms with isotropic thermal displacement parameters that are 1.2 times those of the respective atom. Crystallographic data for the structural analysis have been deposited with the Cambridge Crystallographic Data Centre, CCDC 1063097.

## Results and discussion

Scheme 1 represents the previously reported synthetic pathway followed in the preparation of final compounds **31–45**,[10] and Table 1 shows the structural data of all the intermediate and final compounds synthesized.

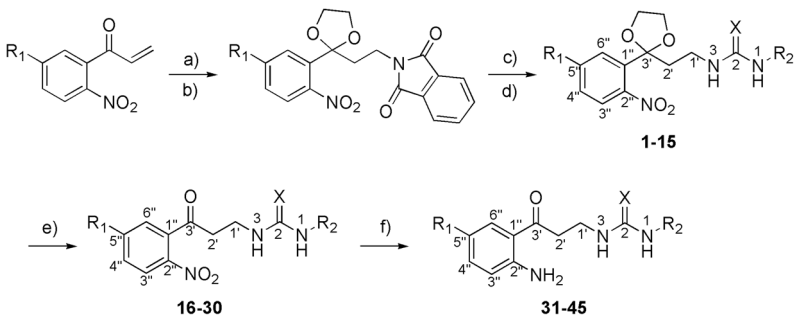
Structural elucidation of these compounds has been made by routine $^1H$ and $^{13}C$ techniques. Nevertheless, a definitive assignment of all signals needs the use of several NMR techniques as follows: (i) DEPT experiments to determine the number of protons attached to each carbon atom; (ii) HSQC spectra to determine the $^{13}C$ resonances of the tertiary, secondary and primary carbons; (iii) HMBC sequences to assign the signals of quaternary carbons via two-bond and three-bond interactions; and (iv) NOESY experiments to determinate the preferred conformation in solution.

Tables 2–4 show the $^1H$ NMR signals of each proton for compounds **1–45**, whereas Tables 5–7 show the corresponding $^{13}C$ NMR chemical shifts for the same molecules. NMR spectra of all compounds were carried out in $CDCl_3$ solution, except for compound **19**, which was registered in $(CD_3)_2CO$, **20** and **37** in $(CD_3)_2SO$ and **26** and **33** in $CD_3OD$. For this reason, some significant variations are observed in the chemical shifts according to the solvent.

The $^1H$ NMR and $^{13}C$ NMR signals of the aromatic ring (H-1″–H-6″, C-1″–C-6″) and C-3′ are similar in the three families of compounds: 1-(2-(2-(5-substituted-2-nitrophenyl)-1,3-dioxolan-2-yl)ethyl)-3-alkylthioureas and alkylureas **1–15**, 1-(3-(5-substituted-2-nitrophenyl)-3-oxopropyl)-3-alkylthioureas and alkylureas **16–30**, and 1-(3-(2-aminophenyl-5-substituted)-3-oxopropyl)-3-alkylthioureas and alkylureas **31–45**. However, signals corresponding to the linear chain (H-1′, H-2′, C-2, C-1′ and C-2′) are influenced by the thiourea or urea residue of each family of compounds. Finally, H-1 and H-3 signals, linked to nitrogen atoms, do not follow the same pattern and sometimes are not visible.

Heteronuclear single-quantum correlation and HMBC experiments were performed on some compounds of each series, and the results of these experiments have been extrapolated to the other compounds. Table 8 shows the HSQC correlations for some representative compounds, whereas Fig. 1 shows the more important connectivities found in the HMBC spectra.

Heteronuclear single-quantum correlation experiments performed on compounds **1**, **6**, **9** and **11** allow the assignment of




**Scheme 1.** General synthetic pathway followed in the preparation of final compounds **31–45**. (a) Phthalimide, NaOMe, DMSO; room temperature (RT), 2 h; (b) $(CH_2OH)_2$, *p*-TsOH; toluene, reflux, 10 h; (c) $NH_2NH_2$, dry EtOH, reflux, 4.5 h; (d) $XCNR_2$, anhyd $CH_2Cl_2$, RT, overnight; (e) HCl, $CH_2Cl_2$, RT, 4 h; (f) Fe/$FeSO_4$, $H_2O$, 95 °C, 3 h.

**Table 2.** $^{1}$H NMR signal assignments of compounds **1–15**

| Compound | H-1 | H-3 | H-1′ | H-2′ | H-3″ | H-4″ | H-5″ | H-6″ | 2×-$OCH_2$– | $OCH_3$ |
|---|---|---|---|---|---|---|---|---|---|---|
| **1** | n.o. | n.o. | 3.67 (m) | 2.48 (t, 5.8) | 7.51 (ddd, 3.3, 4.9, 7.8) | 7.43 (m) | 7.43 (m) | 7.79 (d, 7.8) | 4.03 (m), 3.66 (m) | — |
| **2** | n.o. | n.o. | 3.67 (m) | 2.47 (t, 5.8) | 7.51 (m) | 7.42 (m) | 7.42 (m) | 7.60 (m) | 4.02 (m), 3.67 (m) | — |
| **3** | n.o. | n.o. | 3.68 (m) | 2.47 (t, 5.9) | 7.51 (m) | 7.42 (m) | 7.42 (m) | 7.58 (m) | 4.02 (m), 3.68 (m) | — |
| **4** | 6.43 (bs) | 5.97 (bs) | 3.68 (m) | 2.53 (m) | 7.48 (d, 8.8) | 6.86 (dd, 2.8, 8.8) | — | 7.06 (d, 2.8) | 4.02 (m), 3.68 (m) | 3.85 (s) |
| **5** | 6.51 (bs) | 6.51 (bs) | 3.68 (m) | 2.54 (m) | 7.49 (d, 8.8) | 6.86 (dd, 2.7, 8.8) | — | 7.07 (d, 2.7) | 4.02 (m), 3.68 (m) | 3.85 (s) |
| **6** | 6.38 (bs) | 5.88 (bs) | 3.68 (m) | 2.52 (m) | 7.48 (d, 8.8) | 6.85 (dd, 2.8, 8.8) | — | 7.06 (d, 2.8) | 4.00 (m), 3.68 (m) | 3.85 (s) |
| **7** | n.o. | n.o. | 3.70 (m) | 2.46 (t, 5.9) | 7.58 (m) | 7.40 (m) | — | 7.40 (m) | 4.04 (m), 3.70 (m) | — |
| **8** | n.o. | n.o. | 3.75 (m) | 2.52 (t, 5.9) | 7.45 (m) | 7.45 (m) | — | 7.63 (m) | 4.09 (m), 3.75 (m) | — |
| **9** | n.o. | n.o. | 3.70 (m) | 2.48 (t, 6.0) | 7.40 (m) | 7.40 (m) | — | 7.58 (m) | 4.04 (m), 3.70 (m) | — |
| **10** | n.o. | n.o. | 3.36 (t, 6.0) | 2.36 (t, 6.0) | 7.49 (m) | 7.40 (m) | 7.40 (m) | 7.58 (m) | 4.00 (m), 3.65 (m) | — |
| **11** | 4.58 (bs) | 4.58 (bs) | 3.37 (t, 6.1) | 2.37 (t, 6.1) | 7.49 (m) | 7.40 (m) | 7.40 (m) | 7.58 (m) | 4.01 (m), 3.65 (m) | — |
| **12** | 6.48 (bs) | 6.48 (bs) | 3.36 (m) | 2.72 (t, 5.8) | 7.46 (d, 8.8) | 6.84 (dd, 2.7, 8.8) | — | 7.13 (d, 2.7) | 4.17 (m), 3.63 (m) | 3.84 (s) |
| **13** | 4.90 (bs) | 4.42 (bs) | 3.37 (m) | 2.41 (t, 6.1) | 7.44 (d, 8.8) | 6.84 (dd, 2.7, 8,8) | — | 7.05 (d, 2.7) | 3.98 (m), 3.65 (m) | 3.83 (s) |
| **14** | n.o. | n.o. | 3.29 (t, 5.9) | 2.35 (t, 5.9) | 7.37 (m) | 7.37 (m) | — | 7.58 (m) | 4.03 (m), 3.66 (m) | — |
| **15** | n.o. | n.o. | 3.37 (t, 5.9) | 2.36 (t, 5.9) | 7.37 (m) | 7.37 (m) | — | 7.58 (m) | 4.02 (m), 3.67 (m) | — |

Chemical shifts (in $CDCl_3$) are reported in $\delta$ (in ppm) relative to $CDCl_3$; multiplicities and coupling constants (Hz) are given in parentheses.
$^{1}$H signals for the $R_2$ substituent: **1**, $CH_3$: 2.95 (s); **2**, $CH_2CH_3$: 3.34 (m), 1.23 (t, 7.1); **3**, $CH_2CH_2CH_3$: 3.26 (t, 6.6), 1.62 (m), 0.97 (t, 7.4); **4**, $CH_3$: 2.95 (d, 4.9); **5**, $CH_2CH_3$: 3.34 (m), 1.24 (t, 7.3); **6**, $CH_2CH_2CH_3$: 3.26 (m), 1.62 (m), 0.96 (t, 7.4); **7**, $CH_3$: 2.95 (s); **8**, $CH_2CH_3$: 3.39 (q, 7.2), 1.28 (t, 7.2); **9**, $CH_2CH_2CH_3$: 3.26 (t, 7.5), 1.65 (m), 0.96 (t, 7.5); **10**, $CH_2CH_3$: 3.17 (q, 7.2), 1.12 (t, 7.2); **11**, $CH_2CH_2CH_3$: 3.10 (t, 7.1), 1.51 (m), 0.91 (t, 7.4); **12**, $CH_2CH_3$: 3.30 (q, 7.2), 0.92 (t, 7.2); **13**, $CH_2CH_2CH_3$: 3.09 (m), 1.49 (m), 0.90 (d, 7.4); **14** $CH_2CH_3$: 3.17 (q, 7.4), 1.10 (t, 7.4); **15**, $CH_2CH_2CH_3$: 3.10 (t, 7.4), 1.50 (m), 0.91 (t, 7.4).
n.o., not observable; s, singlet; bs, broad singlet; d, doublet; t, triplet; q, quadruplet; m, multiplet.

**Table 3.** $^{1}$H NMR signal assignments of compounds **16–30**

| Compound | H-1 | H-3 | H-1′ | H-2′ | H-3″ | H-4″ | H-5″ | H-6″ | $OCH_3$ |
|---|---|---|---|---|---|---|---|---|---|
| **16** | 6.51 (bs) | 6.51 (bs) | 4.05 (m) | 3.19 (m) | 8.06 (d, 8.1) | 7.57 (m) | 7.70 (m) | 7.42 (d, 7.5) | — |
| **17** | 6.42 (bs) | 6.42 (bs) | 4.03 (t, 5.5) | 3.17 (t, 5,5) | 8.07 (d, 8.2) | 7.59 (m) | 7.70 (m) | 7.41 (d, 1.2, 7.5) | — |
| **18** | n.o. | n.o. | 4.03 (t, 5.5) | 3.19 (t, 5.5) | 8.07 (dd, 1.2, 8.2) | 7.59 (m) | 7.71 (m) | 7.42 (dd, 1.4, 7.5) | — |
| **19**[a] | 6.96 (bs) | 6.91 (bs) | 3.90 (m) | 3.15 (t, 6.4) | 8.13 (d, 9.2) | 7.16 (dd, 2.8, 9.2) | — | 7.06 (d, 2.8) | 3.96 (s) |
| **20**[b] | 7.50 (bs) | 7.37 (bs) | 3.71 (m) | 3.12 (t, 6.6) | 8.17 (d, 9.1) | 7.21 (dd, 2.7, 9.1) | — | 7.15 (d, 2.7) | 3.92 (s) |
| **21** | 6.34 (bs) | 6.11 (bs) | 4.05 (m) | 3.10 (t, 5.5) | 8.13 (d, 9.2) | 6.98 (dd, 2.7, 9.2) | — | 6.74 (d, 2.7) | 3.90 (s) |
| **22** | n.o. | n.o. | 4.07 (5.3) | 3.17 (t, 5.3) | 8.07 (d, 8.8) | 7.56 (dd, 2.2, 8.8) | — | 7.36 (d, 2.2) | — |
| **23** | n.o. | n.o. | 4.04 (t, 5.4) | 3.18 (t, 5.4) | 8.07 (d, 8.8) | 7.56 (dd, 2.2, 8.8) | — | 7.38 (d, 2,2) | — |
| **24** | n.o. | n.o. | 4.04 (t, 5.5) | 3.18 (t, 5.5) | 8.06 (d, 8.8) | 7.55 (dd, 2.2, 8.8) | — | 7.37 (d, 2.2) | — |
| **25** | n.o. | n.o. | 3.62 (t, 5.6) | 3.03 (t, 5.6) | 8.07 (d, 8.2) | 7.58 (m) | 7.70 (m) | 7.40 (dd, 1.4, 7.5) | — |
| **26**[c] | n.o. | n.o. | 3.58 (m) | 3.37 (m) | 8.17 (dd, 1.2, 8.1) | 7.75 (m) | 7.86 (m) | 7.64 (dd, 1.4, 7.5) | — |
| **27** | n.o. | n.o. | 3.65 (t, 5.6) | 2.99 (t, 5.6) | 8.13 (d, 9.2) | 6.98 (dd, 2.7, 9.2) | — | 6.75 (d, 2.7) | 3.90 (s) |
| **28** | 5.02 (bs) | 4.46 (bs) | 3.53 (t, 6.2) | 3.00 (t, 6.2) | 8.17 (d, 9.2) | 7.14 (dd, 2.7, 9.2) | — | 6.97 (d, 2.7) | 3.94 (s) |
| **29** | 4.87 (bs) | 4.87 (bs) | 3.64 (t, 5.6) | 3.04 (t, 5.6) | 8.06 (d, 8.8) | 7.54 (dd, 2.2, 8.8) | — | 7.36 (d, 2.2) | — |
| **30** | 4.67 (bs) | 4.67 (bs) | 3.64 (t, 5.6) | 3.04 (t, 5.6) | 8.06 (d, 8.8) | 7.54 (dd, 2.2, 8.8) | — | 7.35 (d, 2.2) | — |

[a]Solvent used $(CD_3)_2CO$.

[b]Solvent used $(CD_3)_2SO$.

[c]Solvent used $CD_3OD$.

Chemical shifts (in $CDCl_3$) are reported in $\delta$ (in ppm) relative to $CDCl_3$; multiplicities and coupling constants (Hz) are given in parentheses.

$^{1}$H signals for the $R_2$ substituent: **16**, $CH_3$: 2.96 (s); **17**, $CH_2CH_3$: 3.35 (q, 7.1), 1.20 (t, 7.1); **18**, $CH_2CH_2CH_3$: 3.27 (t, 7.0), 1.61 (m), 0.95 (t, 7.4); **19**, $CH_3$: 2.92 (d, 4.7); **20**, $CH_2CH_3$: 3.31 (m), 1.05 (t, 7.2); **21**, $CH_2CH_2CH_3$: 3.27 (m), 1.61 (m), 0.95 (t, 7.4); **22**, $CH_3$: 2.96 (s); **23**, $CH_2CH_3$: 3.36 (m), 1.26 (t, 7.3); **24**, $CH_2CH_2CH_3$: 3.27 (t, 7.0), 1.63 (m), 0.96 (t, 7.4); **25**, $CH_2CH_3$: 3.17 (q, 7.2), 1.10 (t, 7.2); **26**, $CH_2CH_2CH_3$: 3.11 (t, 7.1), 1.54 (m), 0.99 (t, 7.4); **27**, $CH_2CH_3$: 3.21 (q, 7.2), 1.14 (t, 7.2); **28**, $CH_2CH_2CH_3$: 3.06 (t, 7.0), 1.49 (m), 0.90 (d, 7.5); **29** $CH_2CH_3$: 3.20 (q, 7.2), 1.14 (t, 7.2); **30**, $CH_2CH_2CH_3$: 3.12 (t, 7.0), 1.52 (m), 0.93 (t, 7.4).

n.o., not observable; s, singlet; bs, broad singlet; d, doublet; dd, doublet doublet; t, triplet; q, quadruplet; m, multiplet.

**Table 4.** $^{1}$H NMR signal assignments of compounds **31–45**

| Compound | H-1 | H-3 | H-1′ | H-2′ | H-3″ | H-4″ | H-5″ | H-6″ | –$NH_2$ | $OCH_3$ |
|---|---|---|---|---|---|---|---|---|---|---|
| **31** | 6.51 (bs) | 6.30 (bs) | 4.01 (m) | 3.35 (t, 5.5) | 6.70 (m) | 7.31 (m) | 6.67 (m) | 7.73 (dd, 1.5, 8.4) | 5.30 (bs) | — |
| **32** | 6.48 (bs) | 6.18 (bs) | 3.96 (m) | 3.30 (m) | 6.62 (m) | 7.25 (m) | 6.62 (m) | 7.67 (dd, 1.2, 8.5) | n.o. | — |
| **33**[b] | 6.48 (bs) | 6.18 (bs) | 3.92 (m) | 3.33 (t, 6.3) | 6.79 (dd, 1.1, 8.4) | 7.29 (ddd, 1.4, 7.0, 8.4) | 6.65 (ddd, 1.1, 7.0, 8.4) | 7.85 (dd, 1.4, 8.4) | n.o. | — |
| **34** | 6.47 (bs) | 6.27 (bs) | 3.97 (m) | 3.27 (t, 5.5) | 6.60 (d, 9.0) | 6.95 (dd, 2.9, 9.0) | — | 7.12 (d, 2.9) | 5.95 (bs) | 3.74 (s) |
| **35** | 6.53 (bs) | 6.23 (bs) | 3.96 (m) | 3.25 (t, 5.5) | 6.63 (d, 9.0) | 6.95 (dd, 2.9, 9.0) | — | 7.11 (d, 2.9) | 5.47 (bs) | 3.73 (s) |
| **36** | 6.43 (t, 5.7) | 6.14 (bs) | 3.97 (m) | 3.27 (t, 5.5) | 6.61 (d, 9.0) | 6.96 (dd, 2.9, 9.0) | — | 7.13 (d, 2.9) | 5.95 (bs) | 3.74 (s) |
| **37**[a] | 7.40 (bs) | 7.40 (bs) | 3.62 (m) | 3.16 (t, 6.8) | 6.76 (d, 9.0) | 7.24 (dd, 2.4, 9.0) | — | 7.75 (d, 2.4) | n.o. | — |
| **38** | n.o. | n.o. | 3.96 (t, 5.2) | 3.27 (t, 5.2) | 6.63 (d, 9.0) | 7.21 (d, 9.0) | — | 7.65 (s) | n.o. | — |
| **39** | 5.95 (bs) | 5.95 (bs) | 3.99 (t, 5.5) | 3.26 (m) | 6.69 (d, 8.8) | 7.23 (dd, 2.3, 8.8) | — | 7.67 (d, 2.3) | 5.95 (bs) | — |
| **40** | 4.77 (bs) | 4.77 (bs) | 3.57 (t, 5.5) | 3.15 (m) | 6.62 (m) | 7.24 (ddd, 1.3, 7.1, 8.4) | 6.62 (m) | 7.69 (dd, 1.3, 8.4) | 4.77 (bs) | — |
| **41** | 5.51 (bs) | 5.51 (bs) | 3.56 (t, 5.6) | 3.15 (t, 5.6) | 6.64 (m) | 7.24 (ddd, 1.5, 6.9, 7.9) | 6.64 (m) | 7.67 (dd, 1.5, 8.4) | 5.51 (bs) | — |
| **42** | 5.21 (bs) | 5.21 (bs) | 3.57 (t, 5.5) | 3.14 (m) | 6.73 (d, 9.0) | 6.95 (dd, 2.8, 9.0) | — | 7.16 (d, 2.9) | 5.21 (bs) | 3.74 (s) |
| **43** | 5.02 (bs) | 4.46 (bs) | 3.57 (m) | 3.14 (t, 5.7) | 6.60 (d, 8.9) | 6.94 (dd, 2.8, 8.9) | — | 7.14 (d, 2.8) | 5.95 (bs) | 3.74 (s) |
| **44** | 4.59 (bs) | 4.59 (bs) | 3.58 (t, 5.2) | 3.17 (m) | 6.60 (d, 8.8) | 7.19 (dd, 2.2, 8.8) | — | 7.63 (d, 2.2) | 4.59 (bs) | — |
| **45** | 5.18 (bs) | 5.18 (bs) | 3.58 (t, 5.6) | 3.14 (t, 5.6) | 6.62 (d, 8.8) | 7.19 (dd, 2.4, 8.8) | — | 7.62 (d, 2.4) | 5.18 (bs) | — |

[a]Solvent used $(CD_3)_2SO$.

[b]Solvent used $CD_3OD$.

Chemical shifts (in $CDCl_3$) are reported in $\delta$ (in ppm) relative to $CDCl_3$; multiplicities and coupling constants (Hz) are given in parentheses.

$^{1}$H signals for the $R_2$ substituent: **31**, $CH_3$: 2.90 (s); **32**, $CH_2CH_3$: 3.30 (m), 1.18 (t, 7.2); **33**, $CH_2CH_2CH_3$: 3.37 (m), 1.63 (m), 0.99 (t, 7.4); **34**, $CH_3$: 2.09 (d, 4.9); **35**, $CH_2CH_3$: 3.33 (m), 1.17 (t, 7.2); **36**, $CH_2CH_2CH_3$: 3.21 (m), 1.57 (m), 0.92 (t, 7.4); **37**, $CH_3$: 2.76 (s); **38**, $CH_2CH_3$: 3.33 (m), 1.19 (t, 7.2); **39**, $CH_2CH_2CH_3$: 3.26 (m), 1.58 (m), 0.96 (t, 7.4); **40**, $CH_2CH_3$: 3.16 (m), 1.09 (t, 7.2); **41**, $CH_2CH_2CH_3$: 3.06 (t, 7.1), 1.45 (m), 0.86 (t, 7.4); **42**, $CH_2CH_3$: 3.14 (m), 1.06 (t, 7.2); **43**, $CH_2CH_2CH_3$: 3.07 (q, 6.8), 1.46 (m), 0,87 (t, 7.4); **44** $CH_2CH_3$: 3.17 (m), 1.10 (t, 7.2); **45**, $CH_2CH_2CH_3$: 3.09 (t, 7.2), 1.50 (m), 0.91 (t, 7.4).

n.o., not observable; s, singlet; bs, broad singlet; d, doublet; dd, doublet doublet; ddd, doublet, doublet doublet; t, triplet; q, quadruplet; m, multiplet.

**Table 5.** $^{13}C$ NMR chemical shifts of compounds **1–15**

| Compound | C-2 | C-1′ | C-2′ | C-3′ | C-1″ | C-2″ | C-3″ | C-4″ | C-5″ | C-6″ | 2x$C_{diox}$ | $OCH_3$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | 180.82 | 40.04 | 38.06 | 109.28 | 134.66 | 149.51 | 131.61 | 129.82 | 123.48 | 128.38 | 65.04 | |
| **2** | 180.92 | 40.09 | 38.18 | 109.46 | 134.79 | 149.64 | 131.70 | 129.93 | 123.59 | 128.48 | 65.14 | — |
| **3** | 180.39 | 39.96 | 37.98 | 109.23 | 134.56 | 149.46 | 131.47 | 129.72 | 123.37 | 128.29 | 64.93 | — |
| **4** | 182.19 | 40.35 | 37.97 | 109.54 | 137.65 | 143.01 | 126.24 | 114.28 | 161.94 | 113.38 | 65.14 | 56.17 |
| **5** | 181.98 | 40.31 | 37.95 | 109.43 | 137.42 | 143.14 | 126.22 | 114.29 | 161.72 | 113.33 | 65.12 | 56.14 |
| **6** | 181.34 | 40.28 | 37.94 | 109.54 | 137.61 | 143.05 | 126.19 | 114.27 | 161.92 | 113.38 | 65.13 | 56.16 |
| **7** | 181.66 | 40.06 | 38.12 | 109.06 | 137.21 | 147.85 | 125.18 | 130.00 | 137.91 | 128.59 | 65.33 | — |
| **8** | 180.68 | 39.98 | 38.09 | 109.06 | 137.18 | 147.86 | 125.16 | 129.99 | 137.90 | 128.59 | 65.32 | — |
| **9** | 180.79 | 40.07 | 38.10 | 109.07 | 137.18 | 147.88 | 125.16 | 130.00 | 137.89 | 128.59 | 65.33 | — |
| **10** | 158.50 | 35.66 | 39.37 | 109.51 | 135.15 | 149.66 | 131.47 | 129.68 | 123.38 | 128.63 | 65.07 | — |
| **11** | 158.54 | 35.59 | 39.04 | 109.24 | 135.07 | 149.46 | 131.29 | 129.52 | 123.20 | 128.42 | 64.88 | — |
| **12** | 158.57 | 35.59 | 39.01 | 109.45 | 137.62 | 144.92 | 125.74 | 114.08 | 161.42 | 113.21 | 64.83 | 55.62 |
| **13** | 158.40 | 35.57 | 39.01 | 109.46 | 137.79 | 142.97 | 125.76 | 114.00 | 161.58 | 113.26 | 64.91 | 55.94 |
| **14** | 158.77 | 35.43 | 42.39 | 108.93 | 137.20 | 147.69 | 124.70 | 129.43 | 137.30 | 128.41 | 64.87 | — |
| **15** | 158.78 | 35.25 | 42.39 | 108.89 | 137.26 | 147.61 | 124.66 | 129.48 | 137.35 | 128.44 | 64.97 | — |

$\delta$ (in ppm) relative to $CDCl_3$. $^{13}C$ signals for the $R_2$ substituent: **1**, $CH_3$: 32.81; 30.29; **2**, $CH_2CH_3$: 38.18, 14.28; **3**, $CH_2CH_2CH_3$: 45.60, 22.16, 11.44; **4**, $CH_3$: 30.50; **5**, $CH_2CH_3$: 42.25, 22.65; **6**, $CH_2CH_2CH_3$: 45.78, 22.39, 11.63; **7**, $CH_3$: 30.47; **8**, $CH_2CH_3$:38.80, 14.26; **9**, $CH_2CH_2CH_3$: 45.80, 22.37, 11.66; **10**, $CH_2CH_3$: 35.66, 15.64; **11**, $CH_2CH_2CH_3$: 42.56, 23.26, 11.36; **12**, $CH_2CH_3$: 41.79, 15.47; **13**, $CH_2CH_2CH_3$: 42.49, 23.50, 11.44; **14** $CH_2CH_3$: 35.35, 15.31; **15**, $CH_2CH_2CH_3$: 39.02, 23.34, 11.31.

**Table 6.** $^{13}C$ NMR chemical shifts of compounds **16–30**

| Compound | C-2 | C-1′ | C-2′ | C-3′ | C-1″ | C-2″ | C-3″ | C-4″ | C-5″ | C-6″ | $OCH_3$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **16** | 181.50 | 39.50 | 42.50 | 202.54 | 137.20 | 145.76 | 124.50 | 131.09 | 134.50 | 127.30 | — |
| **17** | 181.54 | 39.71 | 42.51 | 202.64 | 137.23 | 145.91 | 124.81 | 131.19 | 134.61 | 127.45 | — |
| **18** | 180.70 | 39.51 | 42.30 | 202.38 | 137.01 | 145.73 | 124.60 | 130.99 | 134.39 | 127.27 | — |
| **19**[a] | 184.29 | 39.44 | 42.36 | 201.28 | 138.56 | 140.82 | 127.21 | 115.60 | 164.71 | 112.51 | 56.36 |
| **20**[b] | 182.37 | 39.41 | 42.63 | 201.95 | 138.43 | 140.64 | 127.86 | 116.31 | 164.70 | 113.20 | 57.31 |
| **21** | 181.83 | 39.86 | 42.73 | 202.74 | 138.26 | 140.44 | 127.50 | 115.49 | 164.75 | 112.06 | 56.63 |
| **22** | 181.76 | 39.70 | 42.64 | 201.09 | 138.87 | 143.95 | 126.35 | 131.07 | 141.70 | 127.51 | — |
| **23** | 181.43 | 39.64 | 42.64 | 201.15 | 138.86 | 143.96 | 126.34 | 131.07 | 141.68 | 127.53 | — |
| **24** | 181.60 | 39.66 | 42.65 | 201.16 | 138.87 | 143.96 | 126.32 | 131.06 | 141.66 | 127.54 | — |
| **25** | 158.53 | 35.64 | 43.43 | 202.55 | 137.58 | 145.92 | 124.74 | 130.99 | 134.53 | 127.50 | — |
| **26**[c] | 160.00 | 34.82 | 41.66 | 202.03 | 137.25 | 146.22 | 124.29 | 131.01 | 134.21 | 127.64 | — |
| **27** | 158.73 | 35.54 | 43.47 | 202.55 | 138.18 | 140.59 | 127.43 | 115.43 | 164.72 | 112.04 | 56.61 |
| **28** | 160.01 | 34.94 | 43.09 | 202.23 | 138.31 | 140.68 | 126.99 | 115.04 | 164.82 | 112.31 | 55.85 |
| **29** | 159.95 | 36.65 | 44.52 | 202.02 | 140.24 | 145.07 | 127.42 | 132.11 | 142.80 | 128.75 | — |
| **30** | 158.99 | 35.58 | 43.35 | 200.91 | 139.03 | 143.92 | 126.30 | 131.00 | 141.69 | 127.58 | — |

[a]Solvent used $(CD_3)_2CO$.
[b]Solvent used $(CD_3)_2SO$.
[c]Solvent used $CD_3OD$.
$\delta$ (in ppm) relative to $CDCl_3$. $^{13}C$ signals for the $R_2$ substituent: : **16**, $CH_3$: 32.80; **17**, $CH_2CH_3$: 38.75, 14.22; **18**, $CH_2CH_2CH_3$: 45.62, 22.13, 11.43; **19**, $CH_3$: 30.20; **20**, $CH_2CH_3$: 38.93, 15.09; **21**, $CH_2CH_2CH_3$: 45.68, 22.32, 11.64; **22**, $CH_3$: 29.93; **23**, $CH_2CH_3$:38.73, 14.21; **24**, $CH_2CH_2CH_3$: 45.75, 22.33, 11.65; **25**, $CH_2CH_3$: 35.35, 15.61; **26**, $CH_2CH_2CH_3$: 34.86, 23.27, 10.46; **27**, $CH_2CH_3$: 35.88, 15.35; **28**, $CH_2CH_2CH_3$: 41.68, 23.27, 10.46; **29** $CH_2CH_3$: 37.07, 16.52; **30**, $CH_2CH_2CH_3$: 42.92, 23.27, 11.53.

the secondary carbon atoms C-1′ and C-2′ chemical shifts, and the tertiary carbon atoms C-3″, C-4″, C-5″ and C-6″ chemical shifts in the dioxolane derivatives **1–15**. These atoms show signals in ranges of 35.25–40.35 (C-1′), 37.94–42.39 (C-2′), 124.66-131.70 (C-3″), 114.00–130.00 (C-4″), 123.20–161.94 (C-5″) and 113.21–128.63 (C-6″).

Similar HSQC experiments performed on compounds **18**, **21**, **23**, **27** and **30** indicate that the $^{13}C$ NMR signals for the analogue secondary and tertiary carbon atoms in the intermediate nitrophenyl derivatives **16–30** are in the following ranges: 34.82–39.86 (C-1′), 41.66–44.52 (C-2′), 124.29–127.86 (C-3″), 115.04–132.11 (C-4″), 134.21–164.82 (C-5″) and 112.04–128.75 (C-6″).

In the same way, HSQC experiments performed on compounds **32**, **35**, **39**, **41**, **43** and **45** confirm that the peaks corresponding to the analogue secondary and tertiary carbon atoms in the final aminophenyl derivatives **31–45** appear around 35.54–41.11 (C-1′), 38.47–39.93 (C-2′), 117.38–120.05 (C-3″), 123.54–135.68 (C-4″), 116.13–151.54 (C-5″) and 113.23–132.57 (C-6″).

To confirm the signals corresponding to quaternary carbons, HMBC spectra on the intermediate (**1**, **6**, **11**, **18**, **21** and **30**) and final

**Table 7.** $^{13}C$ NMR chemical shifts of compounds **31–45**

| Compound | C-2 | C-1′ | C-2′ | C-3′ | C-1″ | C-2″ | C-3″ | C-4″ | C-5″ | C-6″ | $OCH_3$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **31** | 182.21 | 40.06 | 38.47 | 201.92 | 117.62 | 150.38 | 117.47 | 134.97 | 116.26 | 131.22 | — |
| **32** | 181.34 | 40.19 | 38.70 | 202.14 | 117.77 | 150.60 | 117.65 | 135.16 | 116.42 | 131.43 | — |
| **33**[b] | 182.86 | 41.11 | 39.76 | 202.76 | 118.77 | 152.83 | 118.48 | 135.68 | 116.42 | 132.57 | |
| **34** | 182.41 | 40.31 | 38.87 | 201.61 | 117.51 | 145.44 | 119.13 | 124.60 | 150.51 | 113.24 | 56.30 |
| **35** | 181.36 | 40.18 | 38.93 | 201.69 | 117.89 | 144.74 | 119.39 | 124.44 | 150.74 | 113.30 | 56.29 |
| **36** | 181.56 | 40.29 | 38.89 | 201.67 | 117.51 | 145.45 | 119.13 | 124.61 | 150.52 | 113.23 | 56.30 |
| **37**[a] | 181.43 | 39.99 | 38.82 | 200.61 | 117.76 | 150.52 | 119.67 | 134.73 | 121.65 | 130.84 | — |
| **38** | 181.40 | 40.01 | 38.78 | 201.23 | 118.59 | 148.55 | 119.31 | 135.13 | 121.13 | 130.50 | — |
| **39** | 181.52 | 40.00 | 38.85 | 201.24 | 119.03 | 147.84 | 119.62 | 135.11 | 121.65 | 130.52 | — |
| **40** | 158.82 | 35.54 | 39.41 | 201.96 | 117.91 | 150.16 | 117.38 | 134.61 | 116.13 | 131.20 | — |
| **41** | 158.85 | 35.88 | 39.71 | 202.16 | 118.48 | 149.87 | 117.94 | 134.88 | 116.79 | 131.43 | — |
| **42** | 158.57 | 35.64 | 39.93 | 201.91 | 119.33 | 142.75 | 120.05 | 123.54 | 151.54 | 113.83 | 56.24 |
| **43** | 158.49 | 35.73 | 39.81 | 201.76 | 117.90 | 145.29 | 119.04 | 124.13 | 150.48 | 113.47 | 56.25 |
| **44** | 158.62 | 35.80 | 39.59 | 201.15 | 118.59 | 148.82 | 119.19 | 134.98 | 120.83 | 130.49 | — |
| **45** | 158.88 | 35.86 | 39.56 | 201.06 | 118.63 | 148.70 | 119.28 | 134.99 | 120.93 | 130.45 | — |

[a]Solvent used $(CD_3)_2SO$.

[b]Solvent used $CD_3OD$.

$\delta$ (in ppm) relative to $CDCl_3$. $^{13}C$ signals for the $R_2$ substituent: **31**, $CH_3$: 30.25; **32**, $CH_2CH_3$: 38.70, 14.23; **33**, $CH_2CH_2CH_3$: 39.76, 23.55, 11.66; **34**, $CH_3$: 30.42; **35**, $CH_2CH_3$: 38.93, 14.23; **36**, $CH_2CH_2CH_3$: 45.74, 22.32, 11.66; **37**, $CH_3$: 31.12; **38**, $CH_2CH_3$:38.78, 14.22; **39**, $CH_2CH_2CH_3$: 45.82, 22.35, 11.67; **40**, $CH_2CH_3$: 35.35, 15.38; **41**, $CH_2CH_2CH_3$: 42.62, 23.56, 11.59; **42**, $CH_2CH_3$: 35.55, 15.63; **43**, $CH_2CH_2CH_3$: 42.63, 23.61, 11.58; **44** $CH_2CH_3$: 35.82, 15.49; **45**, $CH_2CH_2CH_3$: 42.80, 23.42, 11.57.

**Table 8.** HSQC correlations found for compounds **1**, **6**, **9**, **11**, **18**, **21**, **23**, **27**, **30**, **33**, **35**, **39**, **41**, **43** and **45**

| $^1H/^{13}C$ | Compound | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **1** | **6** | **9** | **11** | **18** | **21** | **23** | **27** | **30** | **32** | **35** | **39** | **41** | **43** | **45** |
| H-1′ | 3.67 | 3.68 | 3.70 | 3.37 | 4.03 | 4.05 | 4.04 | 3.65 | 3.64 | 3.96 | 3.96 | 3.99 | 3.56 | 3.57 | 3.58 |
| C-1′ | 40.04 | 40.28 | 40.07 | 35.50 | 39.51 | 39.86 | 39.64 | 35.54 | 35.58 | 40.19 | 40.18 | 40.00 | 35.88 | 35.73 | 35.86 |
| H-2′ | 2.48 | 2.52 | 2.48 | 2.37 | 3.19 | 3.10 | 3.18 | 2.99 | 3.04 | 3.30 | 3.25 | 3.26 | 3.15 | 3.14 | 3.14 |
| C-2′ | 38.06 | 37.94 | 38.10 | 39.04 | 42.30 | 42.72 | 42.64 | 43.47 | 43.35 | 38.70 | 38.93 | 38.85 | 39.71 | 39.81 | 39.56 |
| H-3″ | 7.51 | 7.48 | 7.40 | 7.49 | 8.07 | 8.13 | 8.07 | 8.13 | 8.06 | 6.62 | 6.63 | 6.69 | 6.64 | 6.60 | 6.62 |
| C-3″ | 131.61 | 126.19 | 125.16 | 131.29 | 124.60 | 127.50 | 126.34 | 127.43 | 126.30 | 117.65 | 119.39 | 119.62 | 117.44 | 119.04 | 119.28 |
| H-4″ | 7.43 | 6.85 | 7.40 | 7.40 | 7.59 | 6.98 | 7.56 | 6.98 | 7.54 | 7.25 | 6.95 | 7.23 | 7.24 | 6.94 | 7.19 |
| C-4″ | 129.82 | 114.27 | 130.00 | 129.52 | 130.99 | 115.49 | 131.07 | 115.43 | 131.00 | 135.16 | 124.44 | 135.11 | 134.88 | 124.13 | 134.99 |
| H-5″ | 7.43 | | | 7.40 | 7.71 | | | | | 6.62 | | | 6.64 | | |
| C-5″ | 123.48 | | | 123.20 | 134.39 | | | | | 116.42 | | | 116.79 | | |
| H-6″ | 7.79 | 7.06 | 7.58 | 7.58 | 7.42 | 6.74 | 7.38 | 6,75 | 7.35 | 7.67 | 7.11 | 7.67 | 7.67 | 7.14 | 7.62 |
| C-6″ | 128.38 | 113.38 | 128.59 | 128.42 | 127.27 | 112.06 | 127.53 | 112.04 | 127.58 | 131.43 | 113.30 | 130.52 | 131.43 | 113.47 | 130.45 |

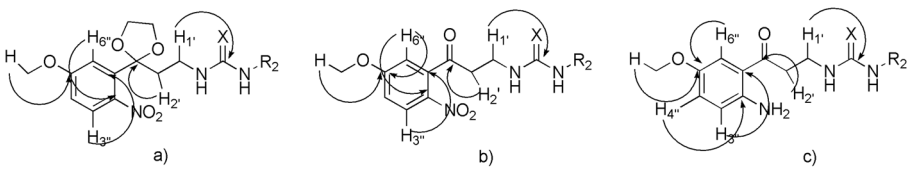



**Figure 1.** Important connectivities found in the HMBC spectra of compounds (a) **1–15**, (b) **16–30** and (c) **31–45**.

(**35**, **39**, **41** and **45**) compounds were recorded (Fig. 1). Correlations in compound **1** between H-6″ ($\delta$ 7.59 ppm) and the $^{13}C$ signal at 149.51 ppm, H-3″ ($\delta$ 7.51 ppm) and the $^{13}C$ signal at 134.66 ppm and H-2″ ($\delta$ 2.48 ppm) and the $^{13}C$ signal at 109.28 ppm allow the unequivocal assignment of C-2″, C-1″ and C-3′, respectively. In addition, compound **6** HMBC analysis indicates a correlation between $OCH_3$ ($\delta$ 3.85) and the $^{13}C$ signal at 161.92 ppm, which can be attributed to C-5″. Also, correlation between H-1′ ($\delta$ 3.37) in compound **11** and the peak at 158.54 ppm shows that this signal corresponds to C-2.

Heteronuclear multiple-bond correlation experiment performed on compound **18** indicates that the signal at $\delta$ 8.07 (H-3″) is correlated with the $^{13}C$ signal at 137.01 ppm, which can be assigned to C-1″, whereas H-6″ signal ($\delta$ 7.42) is correlated with the $^{13}C$ signal

at 145.73 ppm; accordingly, this signal corresponds to C-2″. Furthermore, a correlation between H-1′ signal ($\delta$ 4.03) and $^{13}$C signal at 180.70 ppm has been observed, and this signal can be designated as C-2 of thiourea. Finally, H-2′ ($\delta$ 3.19) is correlated with a signal at $\delta$ 202.38 that can be assigned to C-3′. In addition, similar experiment of compound **21** indicates a correlation between the $OCH_3$ ($\delta$ 3.90) and the $^{13}$C signal at 164.75 ppm that is identified as C-5″. Lastly, HMBC experiment on the urea **30** demonstrates that H-6″ signal ($\delta$ 7.35) is correlated with the $^{13}$C signal at 141.69 ppm, and this signal corresponds to C-5″, whereas correlation between H-1′ ($\delta$ 3,64) and a signal at 158.99 ppm indicates that this signal corresponds to C-2 of urea.

Heteronuclear multiple-bond correlation spectrum of compound **35** denotes that H-3″ ($\delta$ 6.63) and $OCH_3$ ($\delta$ 3.73) are correlated with the $^{13}$C signals at 117.89 and 150.74 ppm, which are assigned to C-1″ and C-5″, respectively. In compound **39**, the signal of $^{13}$C at 147.84 ppm can be assigned to C-2″ because of its correlation with H-4″ ($\delta$ 7.23), whereas H-1′ ($\delta$ 3.99) is correlated with the $^{13}$C signal at 182.52 ppm, and consequently, this peak is identified as C-2 of the thiourea. Also, H-2′ of the urea **41** ($\delta$ 3.15) is correlated with a $^{13}$C signal at 202.16 that is assigned to C-3′. Finally, in compound **45,** the H-6″ ($\delta$ 7.62) and H-1′ ($\delta$ 3.58) are correlated with the $^{13}$C signals at 120.93 and 158.88 ppm that are designed to C-5″ and C-2 of the urea, respectively.

These compounds have been designed from a series of *N*-(3-(2-amino-5-substitutedphenyl)-3-oxopropyl)alkylamide derivatives previously synthesized by our research group,[15] by substitution of the main chain alkylamide for an alkylurea or alkylthiourea ones. The main differences between the $^{13}$C chemical shifts of the previously kynurenamine derivatives[15] and the new kynurenamine-ureas and thioureas are the $^{13}$C signals of the main chain (C-1 atom of the amides and the equivalent C-2 atom of the ureas or thioureas). The amide C-1 atom shows signals in a range of 167.4–176.4, whereas for the equivalent urea C-2 atom, the signals appear in a range of 158.5–160.0, and for the thiourea C-2 atom, the range is 180.7–184.3 ppm, according to similar compounds described in literature.[16,17]

Nuclear Overhauser spectroscopy experiments performed on compound **39** ($R_1$ = Cl, $R_2$ = Pr, X = S) (Fig. 2) demonstrate a correlation between H-6″ and H-2′ and vice versa, which proves that they have close distance in space. These NOE effects are possible if the molecule adopts a conformation where there is an intramolecular hydrogen bond between the carbonyl group of the linear chain and the amino group in 2″-position of the aromatic ring.

To explore the conformational behavior of this molecule in solution, we performed an unrestricted MD simulation (100 ns) of compound **39** in similar conditions to those where NOESY spectra were recorded (box of $CHCl_3$ molecules and 300 K) (Fig. 3(a)). In the three calculated conformational clusters of **39** along the MD simulation, a permanent intramolecular hydrogen bond between the carbonyl oxygen and the amino group of the phenyl ring is observed. In this regard, the distance between $\underline{N}H_2$ and $\underline{O}$=C remains under 2.8 Å along the MD simulation (Fig. 3(b)). As a consequence, hydrogen atoms H-2′ and H-6″ are close in space (2.0–2.5 Å), which is in line with the experimental NOEs. Finally, the major differences between the three groups of conformers are due to the

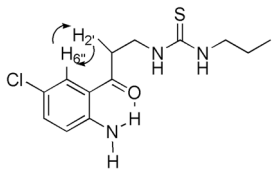



**Figure 2.** Selected NOESY correlations for compound **39**.

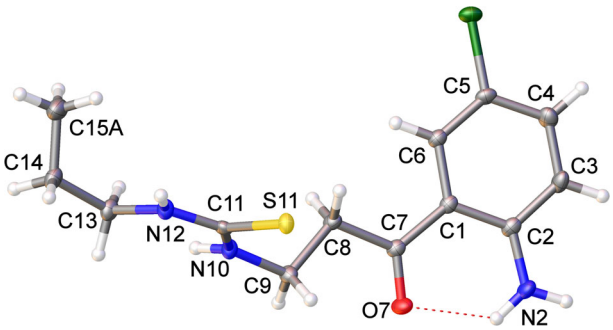



**Figure 4.** Asymmetric unit in the crystal of compound **39**. Only one of the disordered positions of the propyl group is depicted for clarity.

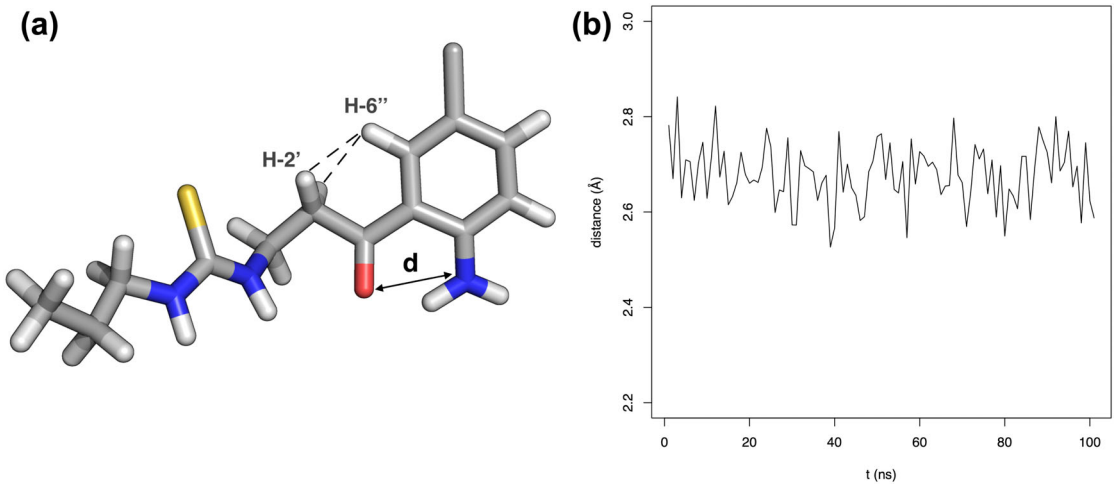



**Figure 3.** Molecular dynamics (MD) simulation of **39** in a box of $CHCl_3$ molecules. (a) Representative structure of the major conformer of **39** and (b) distance evolution between carbonyl oxygen and the amino group calculated along the 100-ns MD simulation. Spatial proximity of H-2″ and H-6″ atoms is indicated as dotted lines.

relative orientation of the thiourea side chain to the ring due to the rotation of C2–C1 bonds in the chain.

Derivative **39** has been crystallized with the object of confirming the preferred conformation of these molecules in solid state, and its 3D structure has been determined using X-ray diffraction. This compound crystallizes in the triclinic system, space group P-1. The asymmetric unit consists of one molecule (Fig. 4) where the mean planes of the aromatic ring and the thiourea moiety defines a dihedral angle of 75.44°. In this conformation, one intramolecular H-bond is observed involving aromatic-$NH_2$ and CO groups (N–H···O: 2.683 A 129.0°). The propyl group is disordered over two alternative positions, with site-occupancy factors 0.52:0.48. In the crystal lattice, pairs of adjacent molecules are symmetrically connected by intermolecular H-bonds that involve aromatic-$NH_2$ and CO groups (N–H···O: 3.184 A 154.0°). The aforementioned pairs of molecules are further associated by additional intermolecular H-bonding interactions where aromatic Cl and thiourea groups are involved, building a 3D H-bonded network.

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