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Experimental NMR and MS study of benzoylguanidines. Investigation of *E/Z* isomerism

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Molecules containing the guanidinic nuclei possess several pharmacological applications, and knowing the preferred isomers of a potential drug is important to understand the way it operates pharmacologically. Benzoylguanidines were synthesized in satisfactory to good yields and characterized by NMR, Electrospray Ionization Mass Spectrometry (ESI-MS) and Fourrier Transform InfraRed Spectroscopy techniques (FTIR). *E/Z* isomerism of the guanidines was studied and confirmed by NMR analysis in solution (^{1}H-^{13}C Heteronuclear Single Quantum Coherence (HSQC) and Heteronuclear Multiple-Bond Correlation (HMBC), ^{1}H-^{15}N HMBC, ^{1}H-^{1}H Correlation Spectroscopy (COSY) and Nuclear Overhauser Effect Spectroscopy (NOESY) experiments) at low temperatures. Compounds with *p*-Cl and *p*-Br aniline moiety exist mainly as *Z* isomer with a small proportion of *E* isomer, whereas compounds with *p*-NO2 moiety showed a decrease in proportion of isomer *Z*. The results are important for the application of these molecules as enzymatic inhibitors. Copyright © 2013 John Wiley & Sons, Ltd.

Supporting information may be found in the online version of this paper.

Keywords: benzoylguanidines; *E/Z* isomerism; NMR experiments; ESI–MS characterization

INTRODUCTION

Guanidine derivatives have been synthesized from different starting materials and reagents.[1] Thioureas have been widely used as the starting materials for guanidine preparation in the presence of different catalysts.[2–12]

Arylthioureas have attracted great attention owing to their diverse biological activities as antiviral agents, herbicides, pesticides, plant growth regulators and chelating agents.[13–17] Guanidinium-based molecules also showed several biological activities such as cardiovascular dilators,[18] antihistaminics,[19] anti-inflammatory agents,[20,21] antidiabetics,[22] antibacterial, antifungal,[23] antiprotozoal, antiparasitic[24] and antiviral.[25,26] Lamy *et al*. have reported 1,3-di-*o*-tolyl guanidines blocking the calcium and potassium channels in dopaminergic neurons.[27] Zanamivir[28] and Peramivir[29] are bioactive guanidines that have activities against all the influenza type A and B viruses.[30]

Benzoylguanidines also form intermolecular and intramolecular H bonds,[2] and this property seems relevant for a potential anticonvulsant activity. Benzoylguanidines are also used as strong and selective inhibitors of Na^{+}/H^{+} exchangers that are integral membrane proteins capable of exchanging intracellular H^{+} for extracellular Na^{+} ions. Alterations in Na^{+}/H^{+} exchange have been implicated in pathophysiological processes such as hypertension, postischemic dysfunction and cellular death. The inhibition of the Na^{+}/H^{+} exchanger during cardiac ischemia and reperfusion has been shown to be beneficial for the preservation of the cellular integrity and functional performance.[31–33]

Synthetic approaches toward substituted *N*-benzoylguanidines have described the formation of intramolecular hydrogen bond, but few studies considered the possibility of these compounds existing as *E/Z* isomers in solution.[2,11,34]

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The present work describes an *E/Z* isomeric study of new benzoylguanidines using NMR spectroscopy in solution at low temperature. All the guanidine compounds were fully characterized by electrospray ionization mass spectrometry (ESI–MS) and Fourier transform infrared spectroscopy (FT-IR) techniques.

EXPERIMENTAL

Materials and methods

Commercial benzoylchloride, ammonium isothiocyanate, 4-nitroaniline, 4-bromoaniline, 4-chloroaniline, benzylamine, furfurylamine, cyclohexylamine, butylamine, 1,8-diazabicyclo[5.4.0]undec-7-ene, triethylamine, penta-hydrated bismuth nitrate and the solvents *N,N*-dimethylformamide, acetonitrile and dichloromethane were used without previous purification. Thin layer chromatography was performed on silica gel plates and visualized using iodine reagent. ^{1}H NMR spectra were obtained at 400.13 MHz. ^{13}C NMR spectra were obtained at 100.61 MHz. ^{15}N NMR spectra were obtained at 40.54 MHz. Chemical shifts for ^{1}H NMR and ^{13}C NMR were referenced to tetramethylsilane. Deuterated dimethyl sulfoxide was assigned to $\delta = 2.49$ ppm for ^{1}H NMR and $\delta = 39.5$ ppm for ^{13}C NMR; all NMR peaks were reported in ppm. Data are presented as follows: chemical shift, multiplicity (s = singlet, d = doublet, dd = doublet of doublet, t = triplet, qua = quadruplet, qu = quintuplet, m = multiplet and br s = broad singlet), integration and coupling constants (in Hertz). EI-MS data was obtained by direct infusion into a GC-MS instrument. The energy used for electron ionization was 70 eV. ESI(+)–MS data were obtained by infusing directly into the ESI source by means of a syringe pump at a flow rate of 10 μL min^{-1}. The molecules display rather clean tandem mass spectra with predominance of their protonated molecules. The ESI–MS/MS experiments show fragment ions formed upon collisions with argon that are fully compatible with their proposed structures. ESI–MS and ESI–MS/MS were acquired using a Quadrupole Time-Of-Flight Mass Spectrometer in the positive ion mode from acetonitrile solutions with 0.1% of formic acid and using the following basic operation conditions: Capillary and cone voltages were set to 3.500 and 45 V, respectively, with a desolvation temperature of 100 °C. FT-IR measurements were performed at 23 °C with 124 scans and 4 cm^{-1} spectral resolution in the form of KBr pellets.

General procedure for the synthesis of 1-benzoyl-3-(4-aryl-substituted) thioureas 1–3[35]

To a solution of ammonium isothiocyanate (0.76 g, 10 mmol) in acetonitrile (20 mL) was added benzoyl chloride (1.40 g, 10 mmol), and the resulting mixture was refluxed for 1 h. After that, the reaction solution was filtered off (for removal of ammonium chloride), and 10 mmol of (4-nitro, 4-bromo or 4-cloro) aniline was added. Then, the reaction mixture was refluxed for an additional 2 h. The crude product was filtered off and washed with ice acetonitrile.

General procedure for the synthesis of N-(3-aminopropyl)-2-azepanone[36]

1,8-Diazabicyclo[5.4.0]undec-7-ene (2.28 g, 15 mmol) and water (0.27 g, 15 mmol) were mixed in a round flask coupled to a reflux condenser, and the mixture was heated for 12 h at 85 °C. The corresponding amino-lactam was obtained in the form of a colorless oil and analyzed by gas chromatography–MS.

General procedure for the synthesis of guanidines 4–17[11]

To a solution of 1 mmol of thioureas **1–3** dissolved in 5 mL of *N,N*-dimethylformamide, 2 mmol of amine, 4 mmol of triethylamine and then 1 mmol of $Bi(NO_3)_3.5H_2O$ were added. The solution became black after a few minutes, and the mixture was heated for 24 h at ~100 °C. After this time, the suspension was filtered off through a pad of Celite, and the pad washed with 20 mL of dichlorometane. The impurity of filtrate was removed with extraction with water (4 × 15 mL), and the organic phase dried over anhydrous $MgSO_4$ and evaporated. The crude residue was crystallized from Et_2O/petroleum ether.

RESULTS AND DISCUSSION

Synthesis

Thioureas **1–3** were synthesized following a reported procedure[35] (Scheme 1) and used as intermediates for the subsequent synthesis of guanidines **4–17** (Scheme 2), using the method described by Cunha *et al.*[11] To form **4–17**, thioureas were reacted with *N*-benzylamine, *N*-furfurylamine, *N*-cyclohexylamine, *N*-butylamine and *N*-(3-aminopropyl)-2-azepanone.

Nuclear magnetic resonance

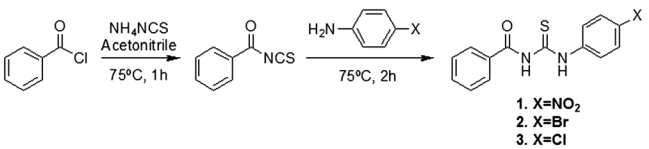
Chemical shifts of ^{1}H and ^{13}C nuclei were unambiguously assigned on the basis of the ^{1}H–^{1}H correlation spectroscopy (COSY), one-bond ^{1}H–^{13}C heteronuclear single quantum coherence (HSQC) and long-range heteronuclear multiple bond correlation (HMBC) NMR measurements. *E/Z* isomerism was investigated by ^{1}H–^{15}N HMBC correlations and Nuclear Overhauser effect spectroscopy (NOESY) experiments at low temperatures. Tables 1–3 show representative chemical shifts in the ^{1}H, ^{13}C and ^{15}N NMR spectra, respectively, for **4–17**.

In the ^{13}C NMR spectra of **1–3**, the chemical shifts of carbonyl and thioureic carbon atoms were observed around 168 and 179 ppm, respectively. The chemical shifts of guanidinic and carbonyl carbon atoms for guanidines were observed around 158 and 177 ppm, respectively. Moreover, the signals for all alkyl (R) groups in **4–17** were observed in the ^{1}H and ^{13}C NMR spectra. The assignment of carbonyl carbon was confirmed through correlation with benzoyl ortho protons in the ^{1}H–^{13}C HMBC NMR spectrum. The other ^{1}H and ^{13}C nuclei were unambiguously confirmed by ^{1}H–^{1}H COSY, ^{1}H–^{13}C HSQC and ^{1}H–^{13}C HMBC NMR spectra.

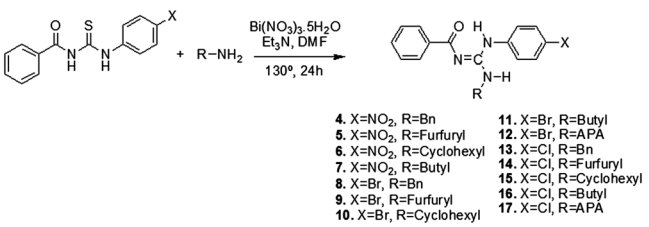
E/Z isomeric study

The chemical shifts of NH hydrogens at room temperature were observed at ~5 and 12 ppm as broad lines with low intensity, which is in agreement with proton exchange and/or substituent motion effects.

The assignment of these hydrogen atoms was performed through ^{1}H and the ^{1}H–^{15}N HMBC NMR experiments at low temperatures (see as example ^{1}H–^{15}N HMBC NMR experiment

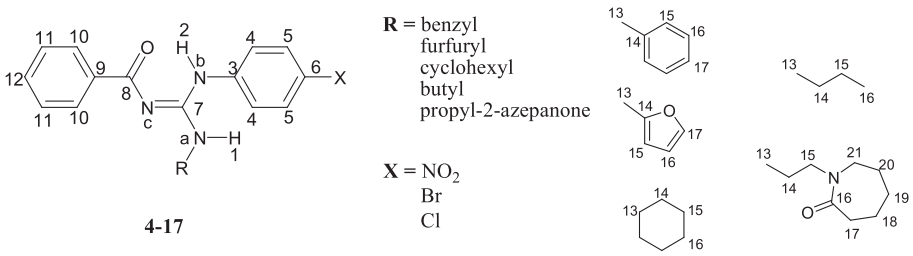


Scheme 1. Synthesis of thioureas **1–3**



Scheme 2. Synthesis of benzoylguanidines **4–17**

Table 1. Representative ^{1}H chemical shifts (ppm) at −60 °C for compounds **4–17**



4-17

Compound	H1	H2	H4	H5	H10	H11	H12	H13	H14	H15
4	5.99	12.63	7.36	8.23	8.29	7.47	7.50	4.92	—	7.42
5	5.94	12.62	7.39	8.32	8.27	7.54	7.58	4.88	—	6.43
6	5.50	12.54	7.36	8.32	8.27	7.51	7.58	3.63	1.07-2.18[a]	
7	5.76	12.51	7.37	8.31	8.28	7.50	7.58	3.68	1.67	1.43
8	5.45	12.15	7.14	7.53	8.29	7.48	7.56	4.85	—	7.41
9	5.43	12.14	7.16	7.56	8.31	7.48	7.52	4.80	—	6.36
10	4.84	12.12	7.17	7.59	8.27	7.52	7.56	4.13	2.12–1.04[a]	
11	4.99	12.09	7.16	7.58	8.29	7.50	7.54	3.58	1.59	1.38
12	7.00	12.29	7.24	7.58	8.28	7.49	7.54	3.53	1.84	3.43
13	5.44	12.16	7.21	7.42	8.29	7.48	7.56	4.85	—	7.40
14	5.45	12.12	7.20	7.38	8.31	7.49	7.56	4.80	—	6.36
15	4.82	12.13	7.23	7.45	8.27	7.51	7.57	4.12	2.13–1.07[a]	
16	5.01	12.05	7.20	7.42	8.27	7.48	7.53	3.56	1.58	1.37
17	6.92	12.27	7.29	7.43	8.28	7.47	7.54	3.53	1.83	3.42

[a]Hydrogens H14, H15 and H16.

Table 2. Representative ^{13}C chemical shifts (ppm) for compounds **4–17**

Compound	C4	C5	C6	C7	C8	C10	C12	C13	C16
4[a]	123.6	125.8	144.1	156.9	177.7	129.0	132.0	45.1	127.9
5[a]	123.5	125.9	143.1	157.8	177.6	129.0	132.0	38.3	110.6
6[a]	123.0	125.8	144.4	156.3	177.5	128.8	131.9	50.6	24.7
7	123.2	125.6	—	—	—	128.8	nm	42.4	13.8
8[a]	127.4	133.1	120.6	158.1	177.5	128.9	131.6	44.8	128.8
9[a]	127.2	133.1	120.4	157.6	177.4	128.9	131.6	37.9	110.4
10	126.9	133.2	120.1	157.7	177.8	129.2	131.4	50.4	25.7
11[a]	127.2	133.1	120.3	158.0	177.3	128.8	131.4	41.1	14.0
12	125.9	132.8	119.5	158.0	177.3	128.9	131.1	37.8	176.8
13	127.1	131.5	—	158.2	177.5	130.1	132.5	44.8	128.9
14[a]	126.9	130.1	132.5	157.7	177.4	128.9	131.5	37.9	110.4
15	126.7	130.3	132.1	157.8	177.8	129.2	131.4	50.4	25.7
16	126.9	130.1	128.6	158.6	177.8	129.1	131.4	41.4	13.9
17[a]	125.4	131.2	—	157.4	177.2	128.7	129.8	37.0	176.9

[a]Measured at −60 °C.

for **11** in Figure 1). The (Na) nitrogen of **11** at 90 ppm shows a doublet correlation indicating ^{1}J coupling correlation with hydrogen H1 at 5.0 ppm and long-range cross-peak with methylene protons H13 and H14 (^{2}J and ^{3}J coupling, respectively) of the butyl substituent and therefore was assigned to alkyl-NH nitrogen. The nitrogen at 107 ppm (Nb) shows a doublet correlation indicating ^{1}J couplings with hydrogen H2 in the 12 ppm region and ^{3}J coupling with aromatic hydrogen H4 at 7.2 ppm corresponding to the ortho protons of the *p*-substituted aromatic ring and was assigned to aryl-NH. Nitrogen at 173 ppm (Nc) did not show ^{1}J coupling, therefore was attributed to amide sp^{2} nitrogen.

^{1}H NMR spectrum at low temperatures (−60 °C) of the compounds allowed observation of another signal attributed to alkyl-NH proton (H1′) as a triplet at ~11 ppm (doublet when R = cyclohexyl) suggesting the existence of *E/Z* isomers. Figures 2 and 3.

Table 3. ^{15}N chemical shifts (ppm) at −60 °C for compounds **9–17**

Compound	R	Na	Nb	Nc
9	Furfuryl	86.87	104.95	172.87
10	Cyclohexyl	105.80	103.72	—
11	Butyl	90.35	103.92	172.94
12	APA	89.08	105.24	173.18
13	Benzyl	91.63	104.21	172.62
14	Furfuryl	86.75	104.74	173.01
15	Cyclohexyl	105.84	103.58	173.22
16	Butyl	90.29	103.74	172.90
17	APA	89.10	105.09	172.83

APA, *N*-(3-aminopropyl)-2-azepanone.

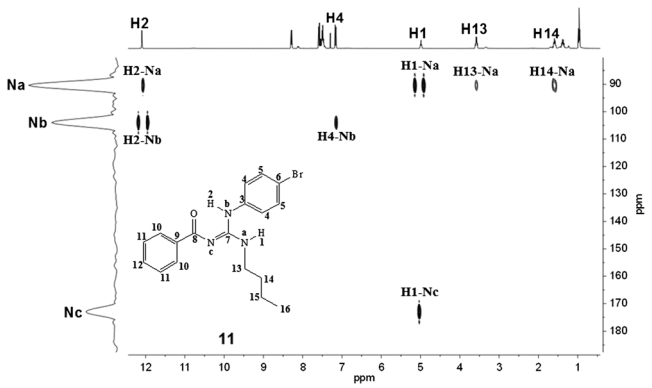


Figure 1. ^{1}H–^{15}N heteronuclear multiple bond correlation NMR experiment at −60 °C for **11**

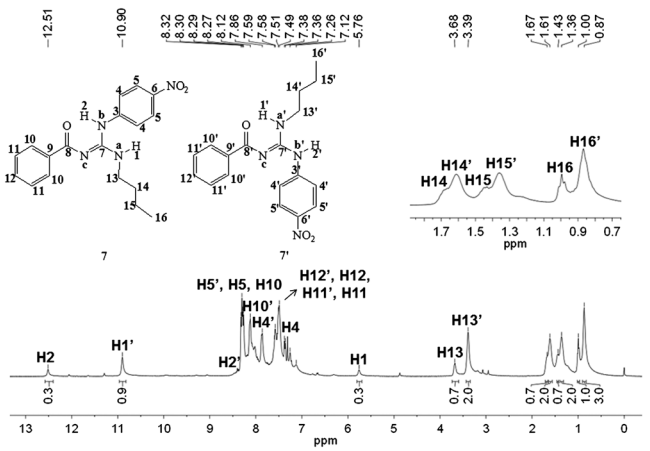


Figure 2. ^{1}H NMR spectrum showing signals of isomers **7** and **7′** at −60 °C

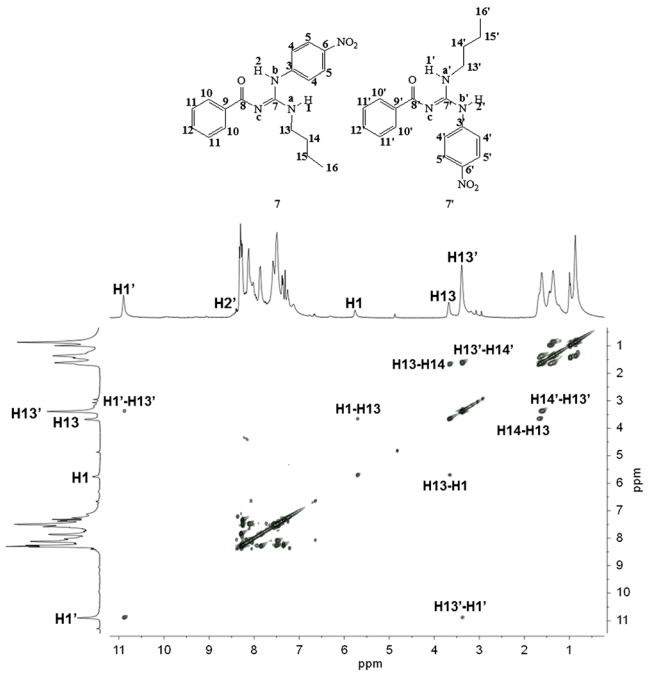
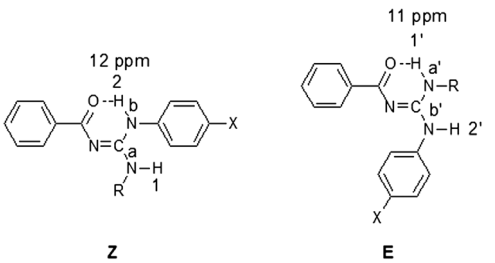


Figure 3. ^{1}H–^{1}H correlation spectroscopy NMR spectrum showing correlations between isomers **7** and **7′** at −60 °C



Scheme 3. Proposed structures for *Z* and *E* isomers

The uncommon chemical shift at ~11 and 12 ppm attributed to respective alkyl-NH (H1′) and aniline (H2) protons (at −60 °C) can be explained as due to intramolecular H-bond formation with carbonyl oxygen atom forming a pseudo six-member ring that could stabilize the *E* and *Z* isomers in solution (Scheme 3).

The (Na′) nucleus of the isomer *E* was observed for some compounds at ~115 ppm in the ^{1}H–^{15}N HMBC NMR experiments (Supplementary material) and showed ^{1}J coupling (Na′) with hydrogen H1′. The NOESY NMR agrees with the presence of *Z* as a main isomer for **8–17** and *E* for **4–7**. Figure 4 shows an example of dipolar couplings observed in NOESY spectrum.

Broad lines were also observed for hydrogens H4 and H10 in all ^{1}H NMR spectra at room temperature, which indicate the occurrence of dynamic process probably caused by an atropisomeric mechanism. Sharp lines were observed at lower temperatures because molecular motion decreases with diminution of temperature, which promotes changes in overall rate of dynamics process as could be expected for atropoisomerism effect. The signal-to-noise ratio increases in the NMR NOESY experiments at lower temperatures because of this restriction in the range of the average motions, making it possible to observe more dipolar couplings with slow protons exchange and molecular motion which support an *E*/*Z* isomerism.

Figure 5(a) shows ^{1}H NMR spectra for **12** at different temperatures. Figure 5(b) represents an expansion of spectral region that contains the signals of –NH-protons for isomer *E*. Figure 5(c) shows signals for H4 and H10 as broad lines at room temperatures and sharp lines at lower ones.

Despite a number of works reporting tautomerism as a main mechanism responsible for broad NMR lines in guanidine

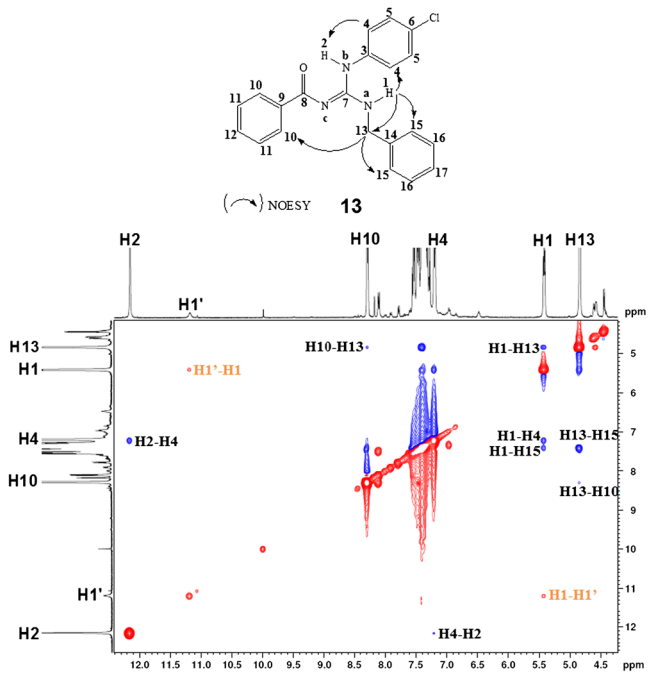


Figure 4. Nuclear Overhauser effect spectroscopy (NOESY) experiment at −60 °C for compound **13**

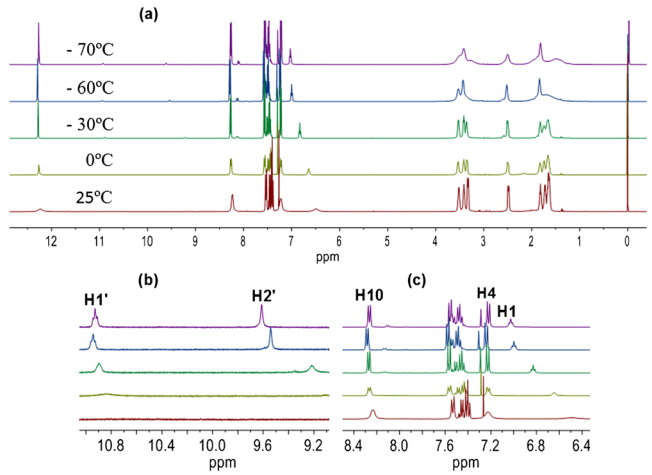


Figure 5. (a) ^{1}H NMR spectra for **12** at different temperatures (25 °C; 0 °C; −30 °C; −60 °C; and −70 °C); (b) Region of –NH hydrogen atoms of isomer E; (c) H4 and H10 regions

systems, the results described in the present manuscript indicate the existence of other effects.

Influence of p-substitution on the *E/Z* ratio

When the ^{1}H NMR spectra at −60 °C of the three different *p*-substituted guanidines (Figure 6) are compared, signals of proton H1′ corresponding to isomer *E* show lower intensities. Isomer *E* could therefore be present in a small proportion and was mainly identified by the multiplicity of the observed signal at ~11 ppm in the ^{1}H NMR spectra.

The compounds with *p*-NO_2 aniline moiety showed a decrease in the *Z* isomer population when compared with *p*-Br and *p*-Cl compounds (Figure 6). This behavior could be explained by strong π-electronic withdrawing effect of nitro group, which

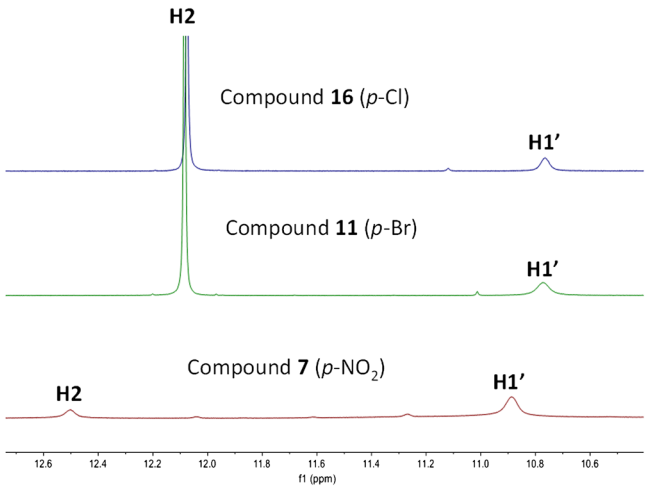


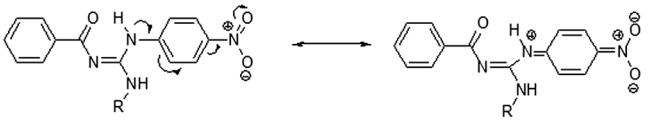
Figure 6. ^{1}H NMR spectra at −60 °C for **7**, **11** and **16**. H2 and H1′ are hydrogen atoms involved in the formation of intramolecular hydrogen bond for isomers *Z* and *E*, respectively

allows the proposal of a zwitterionic resonant structure (Scheme 4). The mesomeric form in Scheme 4 shows an increase of spatial distance between aniline hydrogen atom and carbonyl oxygen. This effect could make the formation of intramolecular hydrogen bond difficult and consequently reduces the *Z* isomers stabilization. *E/Z* isomers proportion was calculated by areas of NH signals in ^{1}H NMR spectra at – 60 °C and is shown in Table 4.

Electrospray ionization mass spectrometry

Benzoylthioureas **1–3** were investigated by ESI(+)–MS, which allowed their detection as intact protonated molecules with corresponding patterns of isotopologue ions. Characterization by tandem mass spectrometry (ESI(+)–MS/MS) showed, as expected from their structures, a major dissociation route leading to the benzoyl cation of *m/z* 105 (main product ion for **1**). The product ion of *m/z* 122 corresponds to neutral loss of *p*-substituted phenyl isothiocyanate and was observed for the protonated molecules of **1–3**, being the main fragment for **2** and **3**. Ions formed by loss of neutral benzoylisothiocyanate moiety (*m/z* 139, 173 and 128, respectively) were also observed for **1–3**, and the product ions of *m/z* 215 and 169 corresponding to neutral loss of benzamide moiety were also observed for **2** and **3**, respectively.

In the ESI(+)–MS, all guanidines **4–17** were identified by the detection of their protonated molecules, which were selected and further fragmented via ESI(+)–MS/MS experiments. Fragmentation of the protonated guanidines **4–17** yielded mainly the product ions of *m/z* 105 and *m/z* 122, corresponding to benzoyl and benzamide cations, respectively. The ion of *m/z* 105 was the most abundant for **7** and *m/z* 122 for **4**. Guanidines **4**, **8** and **13** showed also the benzyl cation of *m/z* 91 as one of the most abundant fragments. For **5**, **9** and **14**, the furfuryl cation of *m/z* 81 was the most abundant fragment. Neutral loss of cyclohexene generating the product ion of *m/z* 285 was observed for **6**, which also showed a major fragment ion of *m/z* 164 corresponding to *p*-nitrophenylcarbodiimide ion. For guanidines **10**, **11**, **15** and **16** (see example for compound **16** in the Figure 7), the product ions of *m/z* 197 and *m/z* 153 were the most abundant (*m/z* 197 for **10** and **11**, and *m/z* 153 for **15** and **16**). These



Scheme 4. Zwitterionic resonant structure for *p*-nitroaniline moiety in compounds **4–7**

Table 4. *E/Z* isomers proportion (%) calculated by ^{1}H NMR at −60 °C

Compound	*p*-group	*Z* isomer	*E* isomer
4	NO_2	53.2	46.8
5	NO_2	55.2	44.8
6	NO_2	38.8	61.2
7	NO_2	22.4	77.6
8	Br	90.1	9.9
9	Br	67.6	32.4
10	Br	90.0	9.1
11	Br	84.7	15.3
12	Br	91.7	8.3
13	Cl	91.7	8.3
14	Cl	93.5	6.5
15	Cl	93.5	6.5
16	Cl	84.0	16.0
17	Cl	91.7	8.3

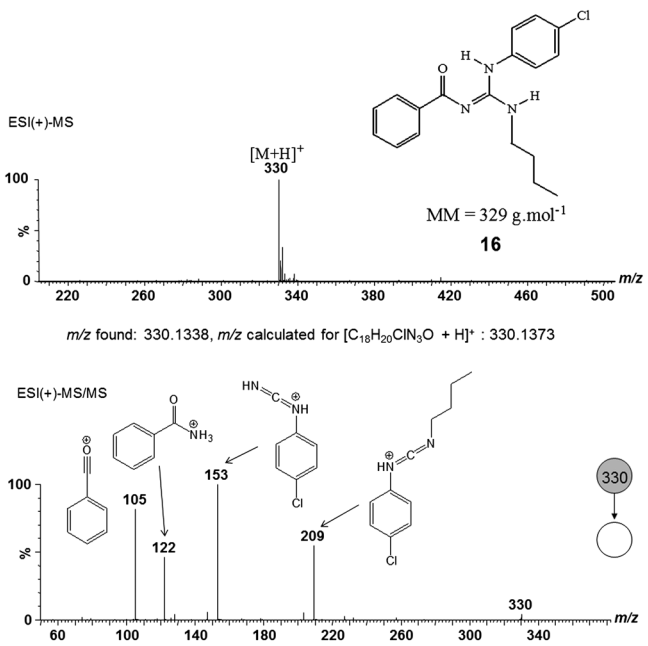
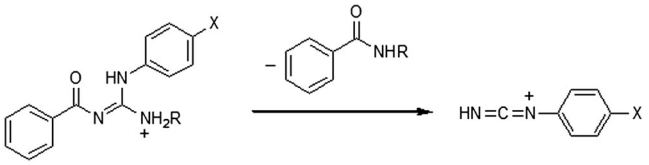


Figure 7. ESI(+)–MS and ESI(+)–MS/MS of protonated **16**

fragments correspond to *p*-substituted phenylcarbodiimides (Scheme 5). Compounds **12** and **17** showed the product ion of *m/z* 154 corresponding to neutral loss of *N*-(*p*-substituted) phenyl-*N*-benzoylguanidine as the most abundant fragment ion.

Fourier transform infrared spectroscopy

The FT-IR spectra of **1–3** showed bands around 3260–3300 cm^{-1} associated to NH groups. The band of the carbonyl ($\nu_{C=O}$) and thiocarbonyl ($\nu_{C=S}$) groups appeared around 1680 and 1260 cm^{-1}, respectively.



Scheme 5. Mechanism proposed for the ion fragment of *m/z* 197 (X = Br, compounds **10** and **11**) and *m/z* 153 (X = Cl, compounds **15** and **16**)

The FT-IR spectra of **4–17** showed, as expected, no absorbance at ~1260 cm^{-1}($\nu_{C=S}$) and a characteristic band at ~1560 cm^{-1} ($\nu_{C=N}$). This supports the transformation of thiourea intermediates to the corresponding guanidines. The bands observed at 3233–3425 cm^{-1} (ν_{N-H}) and 1110 cm^{-1} (ν_{C-N}) also confirm the formation of the guanidines **4–17**.

CONCLUSION

A number of benzoylguanidines are synthesized in satisfactory to good yields. All compounds are well characterized by NMR and MS techniques. *E/Z* isomerism is present as probed by ^{1}H NMR at low temperatures. NMR studies at low temperatures indicate ring rotation or the atropisomerism mechanism as likely effects responsible for dynamic process in guanidine systems. This affirmation was supported by the very small proportions of the tautomeric forms detected at room and low temperature and dipolar couplings observed by NOESY experiments, which in some systems show dipolar connections between the aromatic and aliphatic protons.

SUPPORTING INFORMATION

Synthesis and Isomeric NMR Study of Benzoylguanidines.

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