This is an Accepted Manuscript, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. This Accepted Manuscript will be replaced by the edited, formatted and paginated article as soon as this is available.

You can find more information about Accepted Manuscripts in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal’s standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this Accepted Manuscript or any consequences arising from the use of any information it contains.
Efficient sodium bisulfite-catalyzed synthesis of benzothiazoles and their potential as ureases inhibitors

Débora Pereira de Araujo, Vinicius Stefano Santos Morais, Ângelo de Fátima and Luzia Valentina Modolo

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

In this work we report the successful use of sodium bisulfite as catalyst for the synthesis of 19 benzothiazoles (BZTs) under microwave irradiation with yields from 80% to 100%. BZT-15 was the most active jack bean urease inhibitor exhibiting a mechanism of action typically of mixed inhibitor. Its affinity to bind urease active site is 3-fold higher than that to bind allosteric site(s). The BZTs 2, 8-10, 15 and 16 are described for the first time as soil ureases inhibitors. Overall, these results show the potential of BZTs to be used either as lead compound for the design of drugs for treating urease-induced diseases or as additive in urea-based fertilizers to improve N input in soils used for crop production.

Introduction

Benzothiazoles (BZTs) comprise a class of heterocyclic compounds that bear a benzene ring fused to a thiazole ring at positions 4 and 5. The BZT core is a privileged scaffold as attested by its use for the development of products of agricultural, pharmaceutical and technological interests. For instance, the water soluble l-lysyl amide (phortress) produrg of BZT-5F 203 (Figure 1) is currently on clinical trial in the UK due to the selective antitumor activity of BZT-5F 203 against breast, ovarian and renal human carcinomas. Riluzole (or rilutek); Figure 1 is one of the few drugs approved for the treatment of the neurodegenerative disease amyotrophic lateral sclerosis. BZT-I and BZT-II (Figure 1) exhibit excellent in vitro antifungal activities. Additionally, in vivo assays disclosed that BZT-I is more effective than the commercial antifungal kresoxim-methyl against Sphaerotheca fuliginea and Pseudoperonospora cubensis. A potent inhibitory effect on the ureolytic activity of urease was reported for 2-aminobenzothiazole (BZT-III), exhibiting an IC50 value of 28.88 µg mL-1 (79.7 µM) under the tested experimental conditions.

Several methods were developed for preparing benzofused heterocycles. Among them, two main methodologies have been widely explored for the synthesis of BZTs. The first one is based on the direct reaction of 2-aminothiophenol with aldehydes, ketones, halides or carboxylic acids and derivatives; this is the most used approach for the preparation of BZTs since it develops with no need of sequential multistep reactions to prepare starting materials. The second one involves the intramolecular cyclization of o-substituted-thiobenzamides. The direct approach used to synthesize BZTs presents, however, some bottlenecks that include (i) the use of toxic (e.g. H2O2/HCl, H2O2/Co(NO3)2, H2O2/CAN) and expensive reagents or catalysts (e.g. RuCl3, Ru(PPh3)3(CO)H2/Xanthos, YCl3), (ii) the requirement of long reaction time, (iii) the occurrence of side reactions, (iv) the requirement of laborious workup and/or purification procedures, (v) formation of the desired products in low yields and (vi) the requirement of catalyst synthesis. Therefore, the search for new cost-effective catalysts, in particular, is certainly needed to make the synthesis of BZTs more feasible.

Sodium bisulfite (NaHSO3) has emerged as a cheap catalyst for the formation of C-C and C-O bonds. Its catalytic efficiency for the synthesis of 2,5-substituted 1,3,4-oxadizoles and substituted pyrazoles is also documented. Additionally, NaHSO3 was shown to act as a cocatalyst in phosphorus-free Wittig reactions. Indeed, the use of such inorganic reducing reagent considerably simplifies the product purification since the reaction is carried out in the absence of triphenyl phosphite. Although NaHSO3 was determined to be an efficient catalyst for the synthesis of benzimidazoles under conventional heating, its use as a catalyst for the synthesis of benzothiazoles (BZTs) was not reported up-to-date.

Figure 1. Benzothiazoles (BZTs) of pharmacological interest.
As for the biological activities, the potential of BZTs as ureases inhibitors is still poorly investigated. Urease (EC 3.5.1.5) is a key enzyme for the global nitrogen (N) cycle, occurring in plants, fungi and bacteria. This type of hydrolase speeds up the urea hydrolysis rate to ammonia (NH₃) and carbon dioxide by one hundred trillion folds. The persistence of urease activity in human and animal cells is known to be the cause of some diseases and pathogen infections. For example, urease in human and/or gastrointestinal infections by ureolytic bacteria can cause health complications in humans and animals such as kidney stone formation, pyelonephritis and hepatic encephalopathy. Indeed, major public health issues are related to the Gram-negative bacteria Helicobacter pylori, known to be able to survive under acidic conditions as that of the stomach (pH 2.0).

As a result, uncontrolled proliferation of H. pylori can induce duodenal and gastric ulcers, gastric adenocarcinoma or gastric lymphoma. Then, the use of urease inhibitors may be effective therapies to overcome health issues associated with urease activity.

In addition to health problems, ureases (particularly soil ureases) can negatively affect food production by stimulating N losses to atmosphere from urea-based fertilizers when applied to soil surface. Urea is some of the most used N fertilizers worldwide. Its advantages over other nitrogen fertilizers include high N content (46%), low price, water solubility and easy management. A common practice of soil fertilization is the application of urea to soil surface that, in turn, can lead to over 50% of N losses to atmosphere due to the activity of soil ureases. Thus, the use of urease inhibitors as additive to urea-based fertilizers has received considerable attention as a strategy to overcome N losses in field.

Taking all of these into account, we describe herein the use of sodium bisulfite (NaHSO₃) as an efficient catalyst for the preparation of 19 BZTs under microwave irradiation (MWI). The potential of BZTs synthesized as urease inhibitors of clinical and agricultural interest was also investigated in in vitro and in soil assays.

Results and Discussion

Chemistry: Sodium bisulfite-catalyzed synthesis of benzothiazoles

For the preparation of benzothiazoles (BTZs), we initially carried out several reactions of o-aminophenol with benzaldehyde in the presence of sodium bisulfite (NaHSO₃) as catalyst to find the best reaction conditions to synthesize BTZ-1. The N,N-dimethylacetamide (DMA) was used as solvent in reactions carried out for 15 or 30 min under microwave irradiation (MWI) at different temperatures (80, 100 or 120 °C). The increase of reaction time, accompanied by an increment in temperature, furnished BTZ-1 in much higher yields (Table 1). The maximum reaction yield of 90% was registered upon 30-min-reaction regardless of the use of temperatures higher than 120 °C. It is noteworthy that under the optimal conditions, reactions devoid of NaHSO₃ furnished BTZ-1 in yields lower than 25%. Thus, we demonstrate for the first time that NaHSO₃, a cheap chemical, is a great catalyst for the synthesis of BZTs as it is for providing unsymmetrical ethers, 2,4,5-triphenyl-1H-imidazoles, 2,5-disubstituted 1,3,4-oxadiazoles and substituted pyrazolo[2,3-c]pyrazoles. Additionally, the use of MWI was very effective to speed up BTZ-1 formation since it took 12-fold more time (6 h) to obtain BTZ-1 in comparable yields (85%) from NaHSO₃-catalyzed reactions under conventional heating (120 °C). In fact, MWI has been extensively employed for reducing reaction time and also improving product yields.

Table 1. Effect of time of microwave irradiation (MWI) and temperature on the formation of BTZ-1.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Time (min)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>80 °C</td>
<td>15</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>45</td>
</tr>
<tr>
<td>100 °C</td>
<td>15</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>84</td>
</tr>
<tr>
<td>120 °C</td>
<td>15</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>90</td>
</tr>
</tbody>
</table>

*Reagents and solvent: o-aminophenol (5.0 mmol), benzaldehyde (5.5 mmol), NaHSO₃ (10.0 mmol) and N,N-dimethylacetamide (DMA; 2.0 mL per 5.5 mmol of aldehyde).

Based on these results, 30 min of MWI and temperature of 120 °C were selected as the reaction parameters for further exploring the use of NaHSO₃ as catalyst for obtaining other 18 BZTs. Thus, different (hetero)aromatic aldehydes and cyclohexanecarboxaldehyde were placed to react with o-aminophenol under MWI in the presence of NaHSO₃ to obtain a variety of BZTs (Table 2). The use of NaHSO₃ as catalyst furnished BZTs bearing electron-donating or electron-withdrawing substituents in excellent yields (>80%; Table 2). Previous report described that NaHSO₃ is an efficient catalyst for the synthesis of benzimidazoles under conventional heating, with yields in the range from 13% to 90%. By using a domestic microwave oven and NaHSO₃, benzimidazoles were obtained in yields from 67% to 99%. However, we failed to obtain BZTs in yields higher than 42% under the optimized conditions described for benzimidazoles when using CEM - Microwave-Enhanced Life reactor for organic synthesis instead of a domestic microwave oven. Indeed, results of organic synthesis based on the use of domestic microwave ovens are difficult to be reproducible because such equipment does not allow the reliable control of temperature, irradiation power and the homogeneity of magnetic field. Under our experimental conditions, NaHSO₃-catalyzed reactions yielded BTZ-9 and BTZ-11 in 100% while the use of the catalyst yttrium chloride (YCl₃) furnished these same BZTs in yields lower than 94%. As for the synthesis of BZT-4, BZT-6 and BZT-11, NaHSO₃ furnished higher yields (90-100%) than the copper-DiAmSar complex anchored onto mesoporous SBA-15 silica whose yields ranged from 85-90%. The yield of BZT-14 in NaHSO₃-catalyzed reactions (Table 2)
was 61% and 28% higher than that of bakers’ yeast- and Fe/montmorillonite K-10-catalyzed reactions, respectively.\textsuperscript{20,32} Also, the efficiency of NaHSO\textsubscript{3} for obtaining BZT-4 and BZT-8 (Table 2) was, respectively, \textit{c.a} 25% and 15% higher than that of bakers’ yeast.\textsuperscript{20} The much higher performance of the catalyst NaHSO\textsubscript{3} is also evidenced for the synthesis of BZT-2, BZT-4, BZT-6, BZT-14, BZT-16 and BZT-18 (Table 2) when compared to that of the catalyst sodium dithionite.\textsuperscript{53} Overall, our methodology for the synthesis of BZTs was proven to be efficient and interesting as it uses (\textit{i}) mild conditions and NaHSO\textsubscript{3}, a cheap catalyst, (\textit{ii}) short reaction time (30 min) due to the employment of MWI and (\textit{iii}) an easy procedure (precipitation) for purifying the BZTs.

\textbf{Table 2.} Use of different aldehydes in sodium bisulfite-catalyzed reaction for the synthesis of BZTs under optimized conditions.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BZT-2</td>
<td><img src="BZT2.png" alt="Structure" /></td>
<td>80</td>
</tr>
<tr>
<td>BZT-3</td>
<td><img src="BZT3.png" alt="Structure" /></td>
<td>85</td>
</tr>
<tr>
<td>BZT-4</td>
<td><img src="BZT4.png" alt="Structure" /></td>
<td>90</td>
</tr>
<tr>
<td>BZT-5</td>
<td><img src="BZT5.png" alt="Structure" /></td>
<td>98</td>
</tr>
<tr>
<td>BZT-6</td>
<td><img src="BZT6.png" alt="Structure" /></td>
<td>100</td>
</tr>
<tr>
<td>BZT-7</td>
<td><img src="BZT7.png" alt="Structure" /></td>
<td>100</td>
</tr>
<tr>
<td>BZT-8</td>
<td><img src="BZT8.png" alt="Structure" /></td>
<td>85</td>
</tr>
<tr>
<td>BZT-9</td>
<td><img src="BZT9.png" alt="Structure" /></td>
<td>100</td>
</tr>
<tr>
<td>BZT-10</td>
<td><img src="BZT10.png" alt="Structure" /></td>
<td>92</td>
</tr>
<tr>
<td>BZT-11</td>
<td><img src="BZT11.png" alt="Structure" /></td>
<td>100</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Reagents and solvent: o-aminothiophenol (5.0 mmol), aldehyde (5.5 mmol), NaHSO\textsubscript{3} (10.0 mmol) and \textit{N,N}-dimethylacetamide (DMA; 2.0 mL per 5.5 mmol of aldehyde).

\textit{Biology: Ureases inhibitory activity and kinetic studies}

The effect of 19 BZTs synthesized on ureases activity was first addressed toward jack bean type III urease in reactions containing 10 mM urea and compounds-test at 1.6 mM. The compounds BZT-1, BZT-2, BZT-9 and BZT-15-18 were shown to inhibit the jack bean urease (Figure 2). The BZT-15, the most active synthesized compound, was determined to be as active as hydroxurea (HU; 62% inhibition), a known urease inhibitor, while BZT-16 was found to be as effective as thiourea (TU; 26% inhibition), another known urease inhibitor (Figure 2). The inhibitory activity of BZT-17 was slightly lower than that of TU. The urease inhibition exhibited by the other active BZTs was less expressive being lower than 10% (Figure 2). The results obtained for BZT-9, BZT-10, BZT-12, under our experimental condition, are in agreement with those reported elsewhere.\textsuperscript{54} The results described here for BZT-15 and BZT-16 against jack bean urease contrast those previously reported, in which such BZTs were determined to be almost inactive.\textsuperscript{54} Although the enzymatic reaction conditions were not detailed in the previous report, one can assume they were likely distinct from those described herein. Other BZTs such as 2-amino-6-aryl-benzothiazoles were also active against jack bean urease, exhibiting IC\textsubscripts{50} values in the range from 79.7 to 123.5 µM (concentrations were originally reported in µg mL\textsuperscript{-1}).\textsuperscript{11}
Thus, we selected the most active compound synthesized (BZT-15) to investigate the mechanism of action by which this substance inhibits jack bean urease. Kinetic studies experiments carried out with different concentrations of inhibitor showed that 0.5 mM BZT-15 caused an increment of 30% in urea $K_M$ while an increase of 47% in urea $K_M$ was observed in reactions containing 0.7 mM BZT-15 (Table 3). On the other hand, the urease $V_{max}$ decreased by 14% and 26% in reactions containing BZT-15 at 0.5 mM and 0.7 mM, respectively (Table 3). The concentration-dependent effect of BZT-15 on kinetic parameters of urease, along with the profile of the Lineweaver-Burk plot (Figure 4), indicates that this molecule is a typical mixed inhibitor. This type of inhibitor is known to be able to bind both the free enzyme and the enzyme-substrate complex being, therefore, related to two inhibitor dissociation constants ($K_i$ and $K_{i'}$).\(^\text{55}\) The dissociation constant for the urease-BZT-15 complex ($K_i$) was determined to be 1.02 ± 0.04 mM and the dissociation constant for the urease-urea-BZT-15 complex ($K_{i'}$) was 3.17 ± 0.69 mM. These values indicate that BZT-15 affinity to urease active site is 3-fold higher than that for urease-urea complex. To the best of our knowledge this is the first study about the effect of a BZT on the kinetic parameters of urease.

### Table 3. Effect of BZT-15 on kinetic parameters of jack bean type III urease.

<table>
<thead>
<tr>
<th>BZT-15 (mM)</th>
<th>$K_M$ or $K_{M\text{ (app)}}$ (mM)</th>
<th>$V_{max}$ or $V_{max\text{ (app)}}$ (µmol NH$_3$·min$^{-1}$·mg$^{-1}$ prot)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>3.0</td>
<td>8.8</td>
</tr>
<tr>
<td>0.5</td>
<td>3.9</td>
<td>7.6</td>
</tr>
<tr>
<td>0.7</td>
<td>4.4</td>
<td>6.5</td>
</tr>
</tbody>
</table>

$K_{M\text{ (app)}}$ and $V_{max\text{ (app)}}$ are respectively the apparent $K_M$ and apparent $V_{max}$, i.e., the values of $K_M$ and $V_{max}$ measured for urea and urease in reactions containing BZT-15.

**Figure 2.** Inhibitory effect of benzothiazoles (BZTs) synthesized on jack bean urease. BZTs (1.6 mM) were tested in reactions containing 10 mM urea. Thiourea (TU) and hydroxyurea (HU) were used as reference of urease inhibitors.

**Figure 3.** Michaelis-Menten hyperbola (upper graph) and Lineweaver-Burk plot (lower graph) for jack bean urease as a function of increased concentrations of BZT-15. Reactions containing urea (1-32 mM) were carried out in the absence (BZT-free) or presence of BZT-15 at concentrations indicated.
40 35 10  

p. 109.1, 110.4°C (Lit: points were determined in Gehaka PF 1500 apparatus. Reactions with ureases inhibitor).

2 (DMSO, 200 MHz): δ 7.40, 7.56 (m, 5H), 8.05, 8.11 (m, 4H).

aldehyde (5.5 mmol) were individually added to the mixture.

All chemicals (analytical grade) were obtained from commercial suppliers and used without further purification. 

General procedure for the synthesis of benzothiazoles (BZT-1 - BZT-19): A mixture of NaHSO₃ (10.0 mmol) and dimethylacetamide (DMA) (2 mL) was stirred for 10-20 min. Then, o-aminophenol (5.0 mmol) and different sorts of aldehyde (5.5 mmol) were individually added to the mixture.

Each reaction was subjected to microwave irradiation (MWI) for 30 min at 120 °C in a CEM - Microwave-Enhanced Life reactor, unless otherwise stated. The reaction mixtures were cooled down to room temperature and poured into water to precipitate the product in high purity grade.


Conclusions
This work demonstrates that the use of sodium bisulfite (NaHSO₃) as a catalyst under MWI furnish benzothiazoles (BZTs) in excellent yields. It is demonstrated for the first time that BZT-15 is a mixed-type urease inhibitor that might be used as a lead compound for the design of drugs to treat urease-associated diseases. We also disclose the potential of six BZTs (2, 8, 9, 10, 15 and 16) for use as additive in urea-based fertilizers to improve N availability in soil for crop production.

Experimental section

This journal is © The Royal Society of Chemistry [year]
2-(4-Fluoro)benzothiazole (BZT-8): Yield (85%).

Yellow solid. M. p. 93.8-95.5°C (Lit.: 92.7-98.5°C). 1H NMR (DMSO-d$_6$, 200MHz): δ 7.33-7.57 (m, 4H), 8.02-8.15 (m, 4H). 13C NMR (DMSO-d$_6$, 50MHz): δ 111.6, 116.5, 122.2, 122.7, 125.4, 126.6, 129.4, 129.5, 134.5, 153.4, 161.3, 166.0, 166.3. IR (KBr): 3052, 3028, 2990, 1604, 1520, 1480, 1434, 1228, 986, 836, 756, 728 cm$^{-1}$. LCMS-IT-TOF calculated for C$_{14}$H$_{10}$NOS [M+H]: 242.0634, found: 242.0703.

2-(4-Hydroxyphenyl)benzothiazole (BZT-9): Yield (100%).

Yellow solid. M. p. 224.1-225.9°C (Lit.: 227.0°C). 1H NMR (DMSO-d$_6$, 200MHz): δ 6.95 (sl, 1H), 7.37-7.50 (m, 2H), 7.94-8.04 (m, 4H). 13C NMR (DMSO-d$_6$, 50MHz): δ 116.1, 122.0, 122.2, 123.9, 124.8, 126.3, 129.0, 134.1, 153.7, 160.7, 167.4. IR (KBr): 3054, 2994, 2796, 2590, 1606, 1586, 1432, 1284, 1224, 976, 826, 756, 724 cm$^{-1}$. LCMS-IT-TOF calculated for C$_{13}$H$_{10}$NOS [M+H]: 228.0478, found: 228.0389.

2-(3-Hydroxyphenyl)benzothiazole (BZT-10): Yield (92%).

Yellow solid. M. p. 167.2-168.0°C (Lit.: 169.0°C). 1H NMR (DMSO-d$_6$, 200MHz): 7.00 (d, 1H, $J$ = 7.4 Hz), 7.32-7.55 (m, 5H), 8.07 (t, 2H, $J$ = 8.3 Hz), 9.89 (s, 1H). 13C NMR (DMSO-d$_6$, 50MHz): δ 113.4, 118.0, 118.4, 122.1, 122.8, 128.3, 125.3, 126.5, 130.4, 134.0, 134.3, 153.4, 157.9, 167.3. IR (KBr): 3058, 2698, 2564, 1600, 1480, 1446, 1294, 1270, 1242, 986, 866, 761 cm$^{-1}$. LCMS-IT-TOF calculated for C$_{14}$H$_{10}$NOS [M+H]: 228.0478, found: 228.0398.

2-(2-Hydroxyphenyl)benzothiazole (BZT-11): Yield (100%).

Yellow solid. M. p. 126.7-127.1°C (Lit.: 127.0-128.0°C). 1H NMR (DMSO-d$_6$, 200MHz): 6.97-7.12 (m, 2H), 7.37-7.57 (m, 3H), 8.04-8.17 (m, 3H), 11.67 (sl, 1H). 13C NMR (DMSO-d$_6$, 50MHz): δ 116.9, 118.2, 119.6, 121.9, 122.0, 125.0, 126.4, 128.4, 132.4, 134.1, 151.3, 156.4, 165.3. IR (KBr): 3058, 2996, 2938, 1624, 1590, 1488, 1316, 1272, 1220, 974, 818, 756, 742 cm$^{-1}$. LCMS-IT-TOF calculated for C$_{13}$H$_{10}$NOS [M+H]: 228.0478, found: 228.0388.

2-(2-Thienyl)benzothiazole (BZT-12): Yield (95%).

Yellow solid. M. p. 92.4-94.1°C (Lit.: 92.0-94.0°C). 1H NMR (DMSO-d$_6$, 200MHz): δ 7.20-7.24 (m, 1H), 7.38-7.55 (m, 3H), 7.80-7.86 (m, 2H), 7.97-8.09 (m, 2H). 13C NMR (DMSO-d$_6$, 50MHz): δ 122.2, 122.4, 125.4, 126.6, 128.3, 130.7, 134.1, 136.3, 153.0, 160.8. IR (KBr): 3096, 3056, 1542, 1476, 1312, 1222, 912, 852, 826, 762, 714 cm$^{-1}$. LCMS-IT-TOF calculated for C$_{14}$H$_{10}$NOS$_2$ [M+H]: 218.0093, found: 217.9985.

2-(2-Pyrrolyl)benzothiazole (BZT-13): Yield (98%).

Brown solid. M. p. 153.4-155.3°C (Lit.: 158.0-160.0°C). 1H NMR (DMSO-d$_6$, 200MHz): δ 6.24 (sl, 1H), 6.85 (sl, 1H), 7.05 (sl, 1H), 7.35-7.47 (m, 2H), 7.89-8.01 (m, 2H), 12.12 (s, 1H). 13C NMR (DMSO-d$_6$, 50MHz): δ 110.0, 112.3, 121.5, 121.9, 123.1, 124.3, 125.5, 126.2, 133.4, 153.4, 159.8. IR (KBr): 3154, 3124, 3006, 2860, 1610, 1572, 1488, 1440, 1398, 1102, 912, 762, 740 cm$^{-1}$. LCMS-IT-TOF calculated for C$_{14}$H$_{10}$NOS$_2$ [M+H]: 256.0427, found: 256.0321.

2-Cyclohexyl-benzothiazole (BZT-19): Yield (94%).

Yellow oil (Lit.: yellow oil). 1H NMR (CDCl$_3$, 200MHz): δ 1.27-1.93 (m, 8H), 2.17-2.24 (m, 2H), 3.10 (tt, 1H, $J$ = 11.6/3.6 Hz), 7.28-7.48 (m, 2H), 7.84 (dd, 1H, $J$ = 7.6/0.9 Hz), 7.97 (dd, 1H, $J$ = 8.2/1.0 Hz). 13C NMR (CDCl$_3$, 50MHz): δ 25.7, 26.0, 33.4, 43.4, 121.5, 122.5, 124.4, 125.7, 134.5, 153.1, 177.5. IR (KBr): 3062, 2928, 2852, 1516, 1438, 1314, 1246, 992, 758, 730 cm$^{-1}$. LCMS-IT-TOF calculated for C$_{14}$H$_{10}$NOS$_2$ [M+H]: 218.0998, found: 218.0981.
Urease inhibition assay. The screening for identifying potential urease inhibitors was done using the indophenol method. Each BZT at final concentration of 1.6 mM was incubated in a medium containing 20 mM phosphate buffer (pH 7.0), 1 mM EDTA, 10 mM urea and 12.5 mM of Canavalia ensiformis type III urease. Reactions were maintained at 25 °C for 15 min, followed by addition of 0.5 volume of 1% w/v phenol/5 ppm sodium nitroprusside (SNP) and 0.7 volume of 0.5% w/v NaNH4/0.1% v/v NaOCl solution to interrupt enzyme activity. Reactions were then incubated at 50 °C for 5 min prior to measurement of media absorbance at 630 nm to determine the amount of ammonium (NH4) formed. Hydroxyurea (HU) and thiourea (TU) were used as reference of urease inhibitors. Urease inhibition was determined in terms of percentage of NH4+ formed in compound-test-containing reactions in relation to total urease activity in reactions devoid of inhibitor.

Kinetic assays with jack bean type III urease. The effect of BZTs synthesized on the kinetic parameters of jack bean type III urease was investigated monitoring NH4+ formation in reactions containing or not the BZT at varied concentration and increasing concentrations of urea (1-32 mM). The procedure was similar to that described for assessing the urease inhibition, except that the reaction time was set to 10 min. Kinetic parameters for urease in the absence or presence of BZT were obtained using Hyper32 software. Michaelis-Menten hyperbolas and Lineweaver-Burk plots were obtained using the OriginPro 8 software.

Soil ureases activity assay. The effect activity of soil ureases was assessed using the salicylate method. Clayey dystrophic Red Latosol (oxisol) soil was collected from Brazilian Cerrado (19°28'01.2"S, 44°10'24.5"W). The physical features of the collected soil were 6% coarse sand, 4% fine sand, 12% silt, and 78% clay and chemical analyses showed pH 6.3, 10 mg dm-3 P_Mehlich-1, 129 mg dm-3 K, 4.4 cmol dm-3 Ca, 0.9 cmol dm-3 Mg, 0.1 cmol dm-3 Al, 2.6 cmol dm-3 H+Al, sum of bases of 5.6 cmol dm-3, 68% base saturation, organic matter of 2.5 dag kg-1.

Sieved soil (0.5 g < 2.0 mm particles) were incubated with 72 mM urea in the presence or absence of each BZT (1.6 mM) at 37 °C for 1 h. The activity of soil ureases was stopped by the addition of KCl 1 M/HCl 10 mM solution (5 mL). After 30 min incubation at 25 °C, a supernatant volume was collected and added to a mixture containing 3.4% sodium salicylate, 2.5% sodium citrate, 2.5% sodium tartrate and 120 ppm SNP. Systems were incubated for further 15 min at 25 °C and under darkness. Then, 0.1 volume of 3.0% NaNH4/1.0% NaOCl solution was added to each system following incubation under darkness for 1 h at 25 °C and stirring (600 rpm). Spectrophotometric measurements were carried out at 660 nm to estimate NH4+ formed in the reaction media. The NBPT was used as a reference of urease inhibitor active on soil microbiota.

Acknowledgments
Authors are grateful to Dr. Ivalnildo E. Marriel (EMBRAPA Maize and Sorghum, Brazil) for providing soil samples and related physicochemical characterization data. This work was made possible by the Network for the Development of Novel Urease Inhibitors (www.redniu.org) which is financially supported by the Brazilian agencies CNPq, CAPES and FAPEMIG. LVM and AF are recipients of research fellowships from CNPq.

Notes and references