RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/advances

PAPER

Efficient sodium bisulfite-catalyzed synthesis of benzothiazoles and their potential as ureases inhibitors

Débora Pereira de Araujo,[a,b] Vinícius Stefano Santos Morais,[b] Ângelo de Fátima*[,a] and Luzia Valentina Modolo*[,b]

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.[1-5] For instance, the water soluble L-lysyl amide (phortress) prodrug 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.[6-8] Riluzole (or rilutek®; Figure 1) is one of the few drugs approved for the treatment of the neurodegenerative disease amyotrophic lateral sclerosis.[9] **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*.[10] A potent inhibitory effect on the ureolytic activity of urease was reported for 2-aminobenzothiazole (**BZT-III**), exhibiting an IC_{50} value of 28.88 μg mL^{-1} (79.7 μM) under the tested experimental conditions.[11]

Several methods were developed for preparing benzofused heterocycles.[2,12] Among them, two main methodologies have been widely explored for the synthesis of **BZTs**.[12,13] 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. H_2O_2/HCl,[14] H_2O_2/$Co(NO_3)_2$,[15] H_2O_2/CAN[16]) and expensive reagents or catalysts (e.g. $RuCl_3$,[17] $Ru(PPh_3)_3(CO)H_2$/Xantphos,[18] YCl_3[19]), (*ii*) the requirement of long reaction time,[17,19] (*iii*) the occurrence of side reactions, (*iv*) the requirement of laborious workup and/or purification procedures,[14,17] (*v*) formation of the desired products in low yields[17,18] and (*vi*) the requirement of catalyst synthesis[20,21]. Therefore, the search for new cost-effective catalysts, in particular, is certainly needed to make the synthesis of **BZTs** more feasible.

Sodium bisulfite ($NaHSO_3$) has emerged as a cheap catalyst for the formation of C-C[22] and C-O[23,24] bonds. Its catalytic efficiency for the synthesis of 2,5-substituted 1,3,4-oxadizoles and substituted pyrano[2,3-*c*]pyrazoles is also documented.[25,26] Additionally, $NaHSO_3$ was shown to act as a cocatalyst in phosphorus-free Wittig reactions.[27] 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 $NaHSO_3$ was determined to be an efficient catalyst for the synthesis of benzimidazoles under conventional heating,[28] its use as a catalyst for the synthesis of benzothiazoles (**BZTs**) was not reported up-to-date.

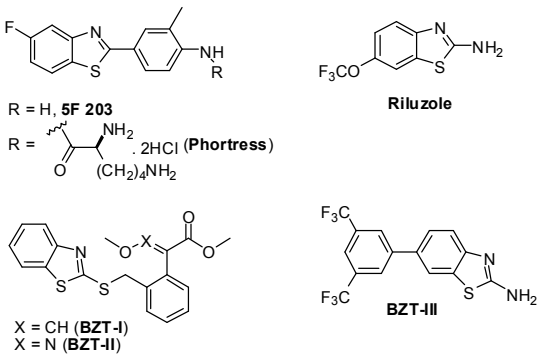


Figure 1. Benzothiazoles (**BZTs**) of pharmacological interest.

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,[29] fungi[30] and bacteria.[31] This type of hydrolase speeds up the urea hydrolysis rate to ammonia (NH_3) and carbon dioxide by one hundred trillion folds.[32] The persistence of urease activity in human and animal cells is known to be the cause of some diseases and pathogen infections. For example, urine and/or gastrointestinal infections by ureolytic bacteria can cause health complications in humans and animals such as kidney stone formation, pyelonephritis and hepatic encephalopathy.[33,34] 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.[7,35] Then, the use of urease inhibitors may be effective therapies to overcome health issues associated with urease activity.[36-40]

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.[41] 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.[41,42] 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.[41]

Taking all of these into account, we describe herein the use of sodium bisulfite ($NaHSO_3$) 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 interested 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*-aminothiophenol with benzaldehyde in the presence of sodium bisulfite ($NaHSO_3$) as catalyst to find the best reaction conditions to synthesize **BZT-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 ºC). The increase of reaction time, accompanied by an increment in temperature, furnished **BZT-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 ºC. It is noteworthy that under the optimal conditions, reactions devoid of $NaHSO_3$ furnished **BZT-1** in yields lower than 25%. Thus, we demonstrate for the first time that $NaHSO_3$, a cheap chemical, is a great catalyst for the synthesis of **BZTs** as it is for providing unsymmetrical ethers, 2,4,5-triphenyl-1*H*-imidazoles, 2,5-disubstituted 1,3,4-oxadiazoles and substituted pyrano[2,3-*c*]pyrazoles.[22-26] Additionally, the use of MWI was very effective to speed up **BZT-1** formation since it took 12-fold more time (6 h) to obtain **BZT-1** in comparable yields (85%) from $NaHSO_3$-catalyzed reactions under conventional heating (120 ºC). In fact, MWI has been extensively employed for reducing reaction time and also improving product yields.[43-47]

Table 1. Effect of time of microwave irradiation (MWI) and temperature on the formation of **BZT-1**[a].

BZT-1

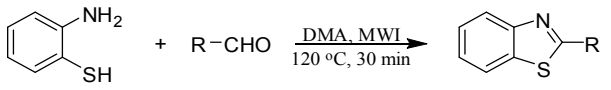
Temperature	Time (min)	Yield (%)
80 ºC	15	6
	30	45
100 ºC	15	27
	30	84
120 ºC	15	42
	30	90

[a]*Reagents and solvent: o*-aminothiophenol (5.0 mmol), benzaldehyde (5.5 mmol), $NaHSO_3$ (10.0 mmol) and *N,N*-dimethylacetamide (DMA; 2.0 mL per 5.5 mmol of aldehyde).

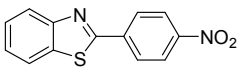
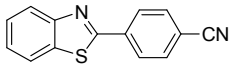
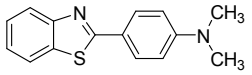
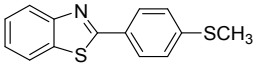
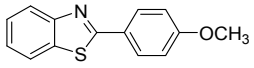
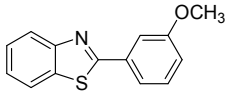
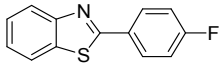
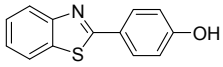
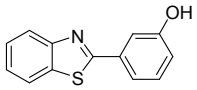
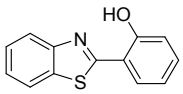
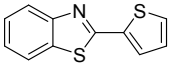
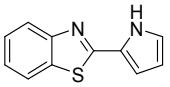
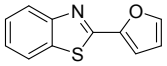
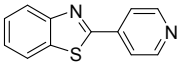
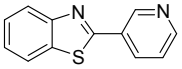
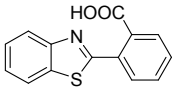
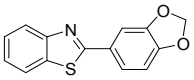
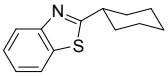
Based on these results, 30 min of MWI and temperature of 120 ºC were selected as the reaction parameters for further exploring the use of $NaHSO_3$ as catalyst for obtaining other 18 **BZTs**. Thus, different (hetero)aromatic aldehydes and cyclohexanecarboxaldehyde were placed to react with *o*-aminothiophenol under MWI in the presence of $NaHSO_3$ to obtain a variety of **BZTs** (Table 2). The use of $NaHSO_3$ as catalyst furnished **BZTs** bearing electron-donating or electron-withdrawing substituents in excellent yields (>80%; Table 2). Previous report described that $NaHSO_3$ is an efficient catalyst for the synthesis of benzimidazoles under conventional heating, with yields in the range from 13% to 90%.[28] By using a domestic microwave oven and $NaHSO_3$, benzimidazoles were obtained in yields from 67% to 99%.[48] However, we failed to obtain **BZTs** in yields higher than 42% under the optimized conditions described for benzimidazoles[48] 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.[49,50] Under our experimental conditions, $NaHSO_3$-catalyzed reactions yielded **BZT-9** and **BZT-11** in 100% while the use of the catalyst yttrium chloride (YCl_3) furnished these same **BZTs** in yields lower than 94%.[51] As for the synthesis of **BZT-4**, **BZT-6** and **BZT-11**, $NaHSO_3$ furnished higher yields (90-100%) than the copper-DiAmSar complex anchored onto mesoporous SBA-15 silica whose yields ranged from 85-90%.[21] The yield of **BZT-14** in $NaHSO_3$-catalyzed reactions (Table 2)

was 61% and 28% higher than that of bakers' yeast- and Fe/montmorillonite K-10-catalyzed reactions, respectively.[20,52] Also, the efficiency of $NaHSO_3$ for obtaining **BZT-4** and **BZT-8** (Table 2) was, respectively, *c.a* 25% and 15% higher than that of bakers' yeast.[20] The much higher performance of the catalyst $NaHSO_3$ 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.[53] Overall, our methodology for the synthesis of **BZTs** was proven to be efficient and interesting as it uses (*i*) mild conditions and $NaHSO_3$, a cheap catalyst, (*ii*) short reaction time (30 min) due to the employment of MWI and (*iii*) an easy procedure (precipitation) for purifying the **BZTs**.

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



(**BZT2-BZT19**)

Compound	Structure	Yield (%)
BZT-2		80
BZT-3		85
BZT-4		90
BZT-5		98
BZT-6		100
BZT-7		100
BZT-8		85
BZT-9		100
BZT-10		92
BZT-11		100
BZT-12		95
BZT-13		98
BZT-14		92
BZT-15		90
BZT-16		90
BZT-17		90
BZT-18		100
BZT-19		94

[a]*Reagents and solvent: o*-aminothiophenol (5.0 mmol), aldehyde (5.5 mmol), $NaHSO_3$ (10.0 mmol) and *N,N*-dimethylacetamide (DMA; 2.0 mL per 5.5 mmol of aldehyde).

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 hydroxyurea (**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.[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.[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_{50} values in the range from 79.7 to 123.5 μM (concentrations were originally reported in μg mL^{-1}).[11]

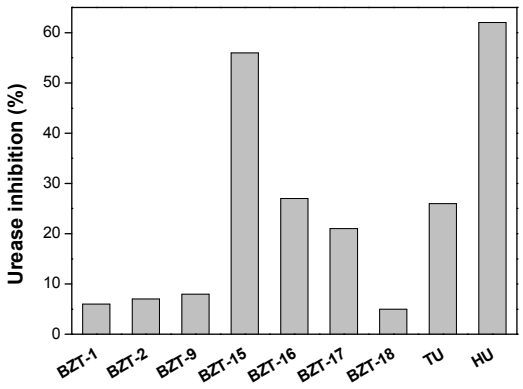


Figure 2. Inhibitory effect of benzothiazoles (**BZTs**) synthesized on jack bean urease. **BZTs** (1.6 mM) were tested in reactions containing 10 mM urea. Thiourea (**TU**) and hydroxyurea (**HU**) were used as reference of urease inhibitors.

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').[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.

BZT-15 (mM)	K_M or $K_{M\ (app)}$ (mM)	V_{max} or $V_{max\ (app)}$ (µmol NH_4^+ min^{-1} mg^{-1} prot)
0.0	3.0	8.8
0.5	3.9	7.6
0.7	4.4	6.5

$K_{M\ (app)}$ and $V_{max\ (app)}$ 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**.

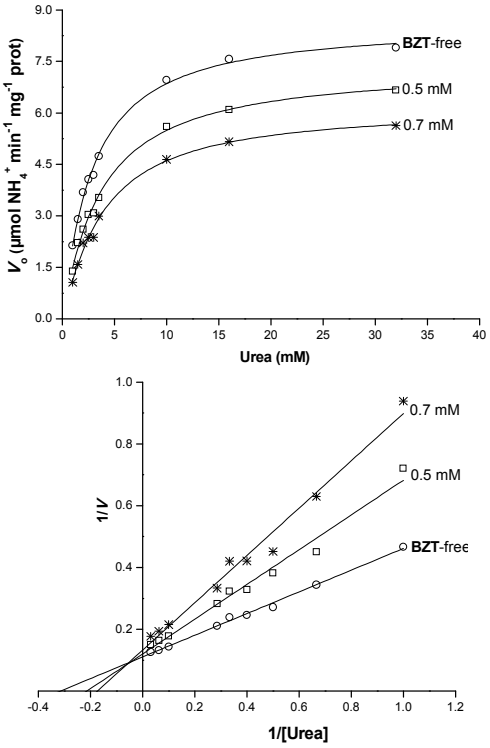


Figure 3. Michaelis-Menten hyperbola (upper graph) and Lineweaver-Burk plot (lower graph) for jack bean urease as a function of increased concentrations of **BZT-15**. Reactions containing urea (1-32 mM) were carried out in the absence (**BZT**-free) or presence of **BZT-15** at concentrations indicated.

Assays using amended soil from Brazilian Cerrado were also performed to investigate the potential of **BZTs** synthesized as urease inhibitors of agricultural interest. Interestingly, 14 out of 19 **BZTs** tested inhibited soil ureases at different extents (Figure 4). Results allowed us to categorize the **BZTs** in four groups: (*i*) group 1 constituted of inhibitors more active than **NBPT** (**BZT-10**); (*ii*) group 2 formed by **BZTs** as active as **NBPT** (**BZT-2**, **BZT-8**, **BZT-9**, **BZT-15** and **BZT-16**); (*iii*) group 3 formed by **BZTs** that inhibited soil ureases by lower than 13% and (*iv*) group 4 that includes non-active **BZTs** (**BZT-6**, **BZT-11**, **BZT-13**, **BZT-17** and **BZT-19**). Surprisingly, **BZT-10**, found to be inactive in *in vitro* assays (Figure 2), was determined to be the most potent soil ureases inhibitor (Figure 4). In the same way, **BZT-17** exhibited *in vitro* urease inhibition comparable to that of the reference **TU**, but fail to inhibit soil ureases (Figures 2 and 4). These can be explained by differences with respect both procedures. The *in vitro* assay with purified urease comprises a less complex system when compared to soil, in which both physicochemical features and the variety of microorganisms present in it may affect at different extents the performance of xenobiotics, as is the case of synthetic urease inhibitors.[56] As a result, interaction of a urease inhibitor with soil matrix may either improve or compromise the efficacy of the inhibitor.

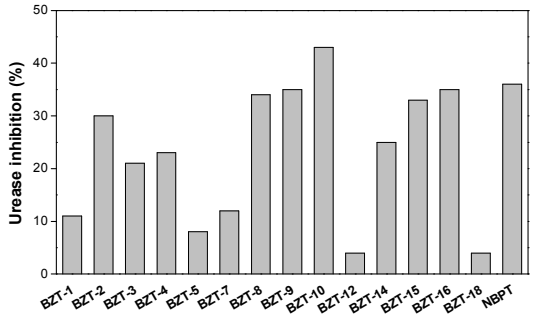


Figure 4. Activity of benzothiazoles (**BZTs**) synthesized on soil ureases. Soil (0.5 g) was incubated with 72 mM urea in the presence of **BZTs** (1.6 mM) or **NBPT** (1.6 mM; reference of soil ureases inhibitor).

Conclusions

This work demonstrates that the use of sodium bisulfite ($NaHSO_3$) 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

All chemicals (analytical grade) were obtained from commercial suppliers and used without further purification. Jack bean type III urease was purchased from Sigma-Aldrich. Uncorrected melting points were determined in Gehaka PF 1500 apparatus. Reactions under microwave irradiation (MWI) were carried out in a CEM - Microwave-Enhanced Life reactor. The ^{1}H nuclear magnetic resonance (NMR) and ^{13}C NMR spectra were recorded on a Bruker Avance DRX/400 or DPX/200. Chemical shift values (δ) were given in parts per million (ppm). Infrared (IR) spectra were recorded on a Spectrometer One Perkin Elmer. Mass spectra were determined on a Shimadzu LCMS-IT-TOF.

General procedure for the synthesis of benzothiazoles (**BZT-1 - BZT-19**): A mixture of $NaHSO_3$ (10.0 mmol) and dimethylacetamide (DMA) (2 mL) was stirred for 10-20 min. Then, *o*-aminethiophenol (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.

2-Phenylbenzothiazole (**BZT-1**): Yield (90%). White solid. M. p. 109.1-110.4°C (Lit:[57] 112.0-113.0ºC). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 7.40-7.56 (m, 5H), 8.05-8.11 (m, 4H). ^{13}C NMR (DMSO-d_6, 50 MHz): δ 121.1, 122.8, 125.4, 126.5, 127.1, 129.2, 131.2, 132.7, 134.4, 153.5, 167.1. IR (KBr): 3062, 1510, 1478, 1432, 1312, 1224, 962, 766, 730, 686 cm^{-1}. LCMS-IT-TOF calculated for $C_{13}H_{10}NS$ $[M+H]^+$: 212.0528, found: 212.0477.

2-(4-Nitrophenyl)benzothiazole (**BZT-2**): Yield (80%). Yellow solid. M. p. 229.5-230.9°C (Lit:[58] 230.0-232.0ºC). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.44-7.60 (m, 2H), 7.97 (d, 1H, J = 7.8 Hz), 8.14 (d, 1H, J = 8.0 Hz), 8.27 (d, 2H, J = 8.9 Hz), 8.36 (d, 2H, J = 8.9 Hz). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 121.8, 124.0, 124.3, 126.2, 126.9, 128.3, 135.6, 139.2, 149.1, 154.2, 164.8. IR (KBr): 3088, 3062, 2990, 2936, 2844, 1606 1596, 1522, 1342, 1312, 1108, 970, 852, 766 cm^{-1}. LCMS-IT-TOF calculated for $C_{13}H_9N_2O_2S$ $[M+H]^+$: 257.0379, found: 257.0325.

2-(4-Cyanphenyl)benzothiazole (**BZT-3**): Yield (85%). Yellow solid. M. p. 167.8-168.9°C (Lit:[58] 169.0-172.0ºC). ^{1}H NMR (DMSO-d_6, 200MHz): δ 7.51 (sl, 2H), 7.92-8.14 (m, 6H). ^{13}C NMR (DMSO-d_6, 50MHz): δ 113.2, 118.2, 122.4, 123.2, 126.1, 126.8, 127.6, 133.1, 134.8, 136.5, 153.3, 165.1. IR (KBr): 3062, 2992, 2226, 1606, 1558, 1480, 1406, 1314, 1250, 968, 838, 764 cm^{-1}. LCMS-IT-TOF calculated for $C_{14}H_9N_2S$ $[M+H]^+$: 237.0481, found: 237.0407.

2-(4-*N*,*N*-Dimethylaminophenyl)benzothiazole (**BZT-4**): Yield (90%). Yellow solid. M. p. 168.2-169.9°C (Lit:[57] 173.0-174.0ºC). ^{1}H NMR ($CDCl_3$, 200MHz): δ 3,03 (s, 6H), 6.73 (d, 2H, J = 8.8 Hz), 7.29 (t, 1H, J = 8.0 Hz), 7.43 (t, 1H, J = 7.6 Hz), 7.83 (d, 1H, J = 8.0 Hz), 7.92 (m, 3H). ^{13}C NMR ($CDCl_3$, 50MHz): δ 40.1, 111.8, 121.3, 121.6, 122.3, 124.1, 125.9, 128.9, 134.7, 152.3, 154.5, 168.7. IR (KBr): 3052, 3028, 2900, 2814, 1610, 1554, 1484, 1430, 1368, 1314, 1226, 1188, 818, 752, 720 cm^{-1}. LCMS-IT-TOF calculated for $C_{15}H_{15}N_2S$ $[M+H]^+$: 255.0950, found: 255.0890.

2-(4-Methylthiophenyl)benzothiazole (**BZT-5**): Yield (98%). Yellow solid. M. p. 146.3-147.6°C (Lit:[59] 142.0-144.0ºC). ^{1}H NMR ($CDCl_3$, 200MHz): δ 2.54 (s, 3H), 7.30-7.53 (m, 4H), 7.89 (d, 1H, J = 7.4 Hz), 7.98-8,07 (m, 3H). ^{13}C NMR ($CDCl_3$, 50MHz): δ 15.3, 121.5, 123.1, 125.1, 126.2, 126.3, 127.8, 130.4, 135.0, 142.8, 154.3, 167.5. IR (KBr): 3052, 2986, 2918, 1654, 1596, 1474, 1434, 1310, 1230, 1094, 960, 814, 758 cm^{-1}. LCMS-IT-TOF calculated for $C_{14}H_{12}N_2S$ $[M+H]^+$: 258.0406, found: 258.0397.

2-(4-Methoxyphenyl)benzothiazole (**BZT-6**): Yield (100%). Beige solid. M. p. 117.7-119.3°C (Lit:[57] 119.0-120.0ºC). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 3.83 (s, 3H), 7.08 (d, 2H, J = 8.4 Hz), 7.37-7.54 (m, 2H), 7.99-8.08 (m, 4H). ^{13}C NMR (DMSO-d_6, 50 MHz): δ 55.3, 114.6, 122.0, 122.4, 124.9, 125.4, 126.4, 128.7, 134.1, 153.6, 161.7, 166.9. IR (KBr): 3062, 2928, 2852, 1594, 1560, 1516, 1438, 1314, 1246, 992, 758, 730 cm^{-1}. LCMS-IT-TOF calculated for $C_{14}H_{12}NOS$ $[M+H]^+$: 242.0634, found: 242.0688.

2-(3-Methoxyphenyl)benzothiazole (**BZT-7**): Yield (100%). Beige solid. M. p. 79.2-81.3°C (Lit:[58] 80.0-81.0ºC). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 3.38 (s, 3H), 7.10-7.16 (m, 1H), 7.41-7.54 (m, 3H), 7.57-7.64 (m, 2H), 8.09 (t, 2H, J = 7.4 Hz). ^{13}C NMR (DMSO-d_6, 50 MHz): δ 55.2, 111.5, 117.2, 119.7, 122.2, 122.8, 125.4, 126.5, 130.4, 134.1, 134.4, 153.4, 159.6, 167.0. IR (KBr): 3078, 3060, 2962, 2934, 2834, 1606, 1582, 1512, 1430,

1314, 1290, 996, 900, 762, 728 cm^{-1}. LCMS-IT-TOF calculated for $C_{14}H_{12}NOS$ $[M+H]^+$: 242.0634, found: 242.0703.

2-(4-Fluorophenyl)benzothiazole (**BZT-8**): Yield (85%). Yellow solid. M. p. 93.8-95.9°C (Lit:[60] 97.2-98.5ºC). ^1H NMR (DMSO-d_6, 200MHz): δ 7.33-7.57 (m, 4H), 8.02-8.15 (m, 4H). ^{13}C NMR (DMSO-d_6, 50MHz): δ 116.1, 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, 968, 836, 756, 728 cm^{-1}. LCMS-IT-TOF calculated for $C_{13}H_9FNS$ $[M+H]^+$: 230.0434, found: 230.0367.

2-(4-Hydroxyphenyl)benzothiazole (**BZT-9**): Yield (100%). Yellow solid. M. p. 224.1-225.9°C (Lit:[61] 227.0ºC). ^1H NMR (DMSO-d_6, 200MHz): δ 6.95 (sl, 1H), 7.37-7.50 (m, 2H), 7.94-8.04 (m, 4H). ^{13}C 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)benzothiazol (**BZT-10**): Yield (92%). Yellow solid. M. p. 167.2-168.0°C (Lit:[61] 169.0ºC). ^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). ^{13}C NMR (DMSO-d_6, 50MHz): δ 113.4, 118.0, 118.4, 122.1, 122.8, 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, 996, 886, 760 cm^{-1}. LCMS-IT-TOF calculated for $C_{13}H_{10}NOS$ $[M+H]^+$: 228.0478, found 228.0398.

2-(2-Hydroxyphenyl)benzothiazol (**BZT-11**): Yield (100%). Yellow solid. M. p. 126.7-127.1°C (Lit:[61] 127.0-128.0ºC). ^1H NMR (DMSO-d_6, 200MHz): δ 6.97-7.12 (m, 2H), 7.37-7.57 (m, 3H), 8.04-8.17 (m, 3H), 11.67 (sl, 1H). ^{13}C 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)benzothiazol (**BZT-12**): Yield (95%). Yellow solid. M. p. 92.4-94.1°C (Lit:[59] 92.0-94.0ºC). ^1H NMR (DMSO-d_6, 200 MHz): δ 7.20-7.24 (m, 1H), 7.38-7.55 (m, 2H), 7.80-7.86 (m, 2H), 7.97-8.09 (m, 2H). ^{13}C NMR (DMSO-d_6, 50 MHz): δ 122.2, 122.4, 125.4, 126.6, 128.6, 129.5, 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 $C_{11}H_8NS_2$ $[M+H]^+$: 218.0093, found: 217.9985.

2-(2-Pyrrolyl)benzothiazol (**BZT-13**): Yield (98%). Brown solid. M. p. 153.4-155.3°C (Lit:[62] 158.0-160.0ºC). ^1H NMR (DMSO-d_6, 200 MHz): 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). ^{13}C NMR (DMSO-d_6, 50 MHz): 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 $C_{11}H_9N_2S$ $[M+H]^+$: 201.0481, found: 201.0402.

2-(2-Furfuryl)benzothiazol (**BZT-14**): Yield (92%). Brown solid. M. p. 102.2-104.0°C (Lit:[57] 102.0-103.0ºC). ^1H NMR (DMSO-d_6, 200 MHz): δ 6.76-6.78 (m, 1H), 7.34-7.57 (m, 3H), 7.99-8.13 (m, 3H). ^{13}C NMR (DMSO-d_6, 50 MHz): δ 111.8, 113.0, 122.2, 122.6, 125.3, 126.7, 133.6, 146.0, 147.9, 153.3, 156.7. IR (KBr): 3144, 3122, 3050, 1598, 1578, 1434, 1246, 1114, 898, 748, 730 cm^{-1}. LCMS-IT-TOF calculated for $C_{11}H_8NOS$ $[M+H]^+$: 202.0321, found: 202.0281.

2-(4-Pyridyl)benzothiazol (**BZT-15**): Yield (90%). Beige solid. M. p. 129.8-131.2°C (Lit:[63] 130.0-132.0ºC). ^1H NMR (DMSO-d_6, 200 MHz): δ 7.59 (sl, 2H), 8.01-8.20 (m, 4H), 8.80 (sl, 2H). ^{13}C NMR (DMSO-d_6, 50 MHz): δ 120.8, 122.5, 123.5, 126.2, 126.9, 134.7, 139.4, 150.8, 153.2, 164.8. IR (KBr): 3052, 3026, 1598, 1588, 1476, 1312, 1214, 980, 820, 756, 704 cm^{-1}. LCMS-IT-TOF calculated for $C_{12}H_9N_2S$ $[M+H]^+$: 213.0481, found: 213.0422.

2-(3-Pyridyl)benzothiazol (**BZT-16**): Yield (90%). Yellow solid. M. p. 124.4-125.7°C (Lit:[64] 137.0-138.0ºC). ^1H NMR (DMSO-d_6, 200 MHz): δ 7.44-7.62 (m, 3H), 8.07-8.18 (m, 2H), 8.41 (d, 1H, J = 7.8 Hz), 8.73-8.75 (m, 1H), 9.25 (sl, 1H). ^{13}C NMR (DMSO-d_6, 50 MHz): δ 122.4, 123.0, 124.2, 125.8, 126.7, 128.8, 134.4, 134.5, 147.6, 151.8, 153.3, 164.4. IR (KBr):3050, 3032, 1586, 1574, 1426, 1310, 1234, 964, 814, 766, 702 cm^{-1}. LCMS-IT-TOF calculated for $C_{12}H_9N_2S$ $[M+H]^+$: 213.0481, found: 213.0399.

2-(2-Carboxyphenyl)benzothiazol (**BZT-17**): Yield (90%). Yellow solid. M. p. 179.3-180.7°C (Lit:[65] 177.0-178.0). ^1H NMR (DMSO-d_6, 200 MHz): δ 7.47-7.80 (m, 6H), 8.03 (d, 1H, J = 7.4 Hz), 8.15 (d, 1H, J = 7.2 Hz). ^{13}C NMR (DMSO-d_6, 50 MHz): δ 122.1, 122.9, 125.4, 126.4, 129.2, 130.4, 131.0, 132.3, 133.2, 135.5, 153.1, 166.3, 168.7. IR (KBr): 3066, 2866, 2756, 2582, 2466, 1702, 1592, 1438, 1318, 1254, 1200, 976, 770, 752 cm^{-1}. LCMS-IT-TOF calculated for $C_{14}H_{10}NO_2S$ $[M+H]^+$: 256.0427, found: 256.0321.

2-(1,3-Benzodioxol-5-yl)benzothiazol (**BZT-18**): Yield (100%). Yellow solid. M. p. 118.4-119.9°C (Lit:[66] 125.0-126.0ºC). ^1H NMR ($CDCl_3$, 200 MHz): δ 6.03 (s, 2H), 6.88 (d, 1H, J = 8.4 Hz), 7.25-7.50 (m, 2H), 7.55-7.60 (m, 2H), 7.85 (d, 1H, J = 7.6 Hz), 8.02 (d, 1H, J = 7.6 Hz). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 101.7, 107.5, 108.6, 121.4, 122.4, 122.9, 124.9, 126.2, 128.0, 134.8, 148.3, 150.0, 154.1, 167.5. IR (KBr): 3058, 2996, 2908, 2784, 1604, 1512, 1474, 1442, 1256, 1032, 928, 882, 806, 756, 728 cm^{-1}. LCMS-IT-TOF calculated for $C_{14}H_{10}NO_2S$ $[M+H]^+$: 256.0427, found: 256.0383.

2-Cyclohexyl-benzothiazol (**BZT-19**): Yield (94%). Yellow oil (Lit:[67] yellow oil). ^1H NMR ($CDCl_3$, 200 MHz): δ 1.27-1.93 (m, 8H), 2.17-2.24 (m, 2H), 3.10 (tt, 1H, J = 11.6/3.6), 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). ^{13}C NMR ($CDCl_3$, 50 MHz): 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 $C_{13}H_{16}NS$ $[M+H]^+$: 218.0998, found:

218.0970.

Urease inhibition assay. The screening for identifying potential urease inhibitors was done using the indophenol method.[68] 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 mU 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 NaOH/0.1% v/v NaOCl solution to interrupt enzyme activity. Reactions were then incubated at 50 °C for 5 min prior the measurement of media absorbance at 630 nm to determine the amount of ammonium (NH_4^+) formed. Hydroxyurea (**HU**) and thiourea (**TU**) were used as reference of urease inhibitors. Urease inhibition was determined in terms of percentage of NH_4^+ 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 NH_4^+ 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.[69] 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 $cmol_c$ dm^{-3} Ca, 0.9 $cmol_c$ dm^{-3} Mg, 0.1 $cmol_c$ dm^{-3} Al, 2.6 $cmol_c$ dm^{-3} H+Al, sum of bases of 5.6 $cmol_c$ 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 f or further 15 min at 25 °C and under darkness. Then, 0.1 volume of 3.0% NaOH/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 NH_4^+ 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. Ivanildo 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

[a]*Grupo de Estudos em Química Orgânica e Biológica (GEQOB), Departamento de Química, Instituto de Ciências Exatas, Universidade Federal de Minas Gerais, Av. Pres. Antônio Carlos, 6627, Pampulha, Belo Horizonte, MG, Brazil, 31270-901; E-mail: adefatima@qui.ufmg.br.*

[b]*Grupo de Estudos em Bioquímica de Plantas (GEBioPlan), Departamento de Botânica, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Av. Pres. Antônio Carlos, 6627, Pampulha, Belo Horizonte, MG, Brazil, 31270-901; E-mail: lvmodolo@icb.ufmg.br.*

† Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/b000000x/

1 P. Hrobárik, I. Sigmundová, P. Zahradník, P. Kasák, V. Arion, E. Franz and K. Clays, *J. Phys. Chem. C*, 2010, **114**, 22289-22302.
2 V. Facchinetti, R. R. Reis, C. R. B. Gomes and T. R. A. Vasconcelos, *Mini-Rev. Org. Chem.*, 2012, **9**, 44-53.
3 M. N. Noolvi, H. M. Patel and M. Kaur, *Eur. J. Med. Chem.*, 2012, **54**, 447-462.
4 M. Henary, S. Paranjpe and E. A. Owens, *Heterocycl. Commun.*, 2013, **19**, 1-11.
5 R. S. Keri, M. R. Patil, S. A. Patil and S. Budagumpi, *Eur. J. Med. Chem.*, 2015, **89**, 207-251.
6 I. Fishtner, A. Monks, C. Hose, M. F. G. Stevens and T. D. Bradshaw, *Breast Cancer Res. Treat.*, 2004, **87**, 97-107.
7 C. G. Mortimer, G. Wells, J. -P. Crochard, E. L. Stone, T. D. Bradshaw, M. F. G. Stevens and A. D. Westwell, *J. Med. Chem.*, 2006, **49**, 179-185.
8 S. Aiello, G. Wells, E. L. Stone, H. Kadri, R. Bazzi, D. R. Bell, M. F. G. Stevens, C. S. Matthews, T. D. Bradshaw and A. D. Westwell, *J. Med. Chem.*, 2008, **51**, 5135-5139.
9 A. Cifra, G. L. Mazzone and A. Nistri, *Neurosci.*, 2012, **19**, 137-144.
10 W. Huang, P. -L. Zhao, C. -L. Liu, Q. Chien, Z. -M. Liu and G. -F. Yang, *J. Agric. Food Chem.*, 2007, **55**, 3004-3010.
11 Y. Gull, N. Rasool, M. Noreen, F. -H. Nasim, A. Yaqoob, S. Kousar, U. Rashid, I. H. Bukhari, M. Zubair and M. S. Islam, *Molecules*, 2013, **18**, 8845-8857.
12 N. P. Prajapati, R. H. Vekariya, M. A. Borad and H. D. Patel, *RSC Adv.*, 2014, **4**, 60176-60208.
13 A. Gupta and S. Rawat, *J. Curr. Pharm. Res.*, 2010, **3**, 13-23.
14 H. Y. Guo, J. C. Li and Y. L. Shang, *Chin. Chem. Lett.*, 2009, **20**, 1408-1410.
15 P. S. Chandrachood, D. R. Garud, T. V. Gadakari, R. C. Torane, N. R. Deshpande, R. V. Kashalkar, *Acta Chim. Slov.*, 2011, **58**, 367-371.
16 K. Bahrami, M. M. Khodaei and F. Naali, *J. Org. Chem.*, 2008, **73**, 6835-6837.
17 X. Fan, Y. Wang, Y. He, X. Zhang and J. Wang, *Tetrahedron Lett.*, 2010, **51**, 3493-3496.
18 A. J. Blacker, M. M. Farah, M. I. Hall, S. P. Marsden, O. Saidi and J. M. J. Williams, *Org. Lett.*, 2009, **11**, 2039-2042.
19 L. Y. Fan, Y. H. Shang, X. X. Li and W. J. Hua, *Chin. Chem. Lett.*, 2015, **26**, 77-80.
20 G. -F. Chen, H. -M. Jia, L. -Y. Zhang, B. -H. Chen and J. -T. Li. *Ultrason. Sonochem.*, 2013, **20**, 627-632.
21 M. Mohammadi, G. R. Bardajee and N. N. Pesyan, *RSC Adv.*, 2014, **4**, 62888-62894.
22 J. N. Sangshetti, N D. Kokare, S. A. Kotharkar, D. B. Shinde, *Monatsh. Chem.*, 2008, **139**, 125-127.
23 H. Wang, X. Zhu, Y. Lu, Y. Li and X. Gao, *Chin. J. Chem.*, 2011, **29**, 1180-1184.
24 J. L. Yu, H. Wang, K. F. Zou, J. R. Zhang, X. Gao, D. W. Zhang and Z. T. Li, *Tetrahedron*, 2013, **69**, 310-315.
25 J. N. Sangshetti, A. R. Chabukswar, D. B. Shinde, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 444-448.

26 S. N. Darandale, J. N. Sangshetti and D. B. Shinde, *J. Korean Chem. Soc.*, 2012, **56**, 328-333.
27 Z. Z. Huang, S. Ye, W. Xia and Y. Tang, *Chem. Commun.*, 2001, 1384-1385.
28 H. F. Ridley, R. G. W. Spickett and G. M. Timmis, *Smith Kline and French Laboratories Ltd. and Chester Beatty Research Institute*, 1965, **2**, 453-456.
29 C. Follmer, *Phytochemistry*, 2008, **69**, 18-28.
30 H. L. Mobley and R. P. Hausinger, *Microbiol. Mol. Biol. Rev.*, 1995, **59**, 451-480.
31 R. A. Burne and Y. Y. M. Chen, *Microbes Infec.*, 2000, **2**, 533-542.
32 B. Krajewska, *J. Mol. Catal. B Enzym.*, 2009, **59**, 9-21.
33 M. J. Maroney and S. Ciurli, *Chem. Rev.*, 2014, **114**, 4206-4228.
34 J. L. Boer, S. B. Mulrooney and R. P. Hausinger, *Arch. Biochem. Biophys.*, 2014, **544**, 142-152.
35 H. M. S. Algood, T. L. Cover, *Clin. Microbiol. Rev.*, 2006, **19,** 597-613.
36 T. -W. Woo, M. -S. Chang, Y. -K. Chung, K. -B. Kim, S. -K. Sohn, S. -G. Kim and W. -S. Choi, *Arch. Pharm, Res.*, 1998, **21**, 6-11.
37 J. S. Williamson, *Curr. Pharm. Des.*, 2001, **7**, 357-394.
38 C. Follmer, *J. Clin. Pathol.*, 2010, **63**, 424-430.
39 H. Azizian, F. Nabati, A. Sharifi, F. Siavoshi, M. Mahdavi and M. Amanlou, *J. Mol. Model.*, 2012, **18**, 2917-2927.
40 L. V. Modolo, A. X. Souza, L. P. Horta, D. P. Araujo and A. de Fátima, *J. Adv. Res.*, 2015, **6**, 35-44.
41 E. Artola, S. Cruchaga, I. Ariz, J. F. Moran, M. Garnica, F. Houdusse, J. M. G. Mina, I. Irigoyen, B. Lasa and P. M. A. Tejo, *Plant Growth Regul.*, 2011, **63**, 73-79.
42 K. C. Cameron, H. J. Di and J. L. Moir, *Ann. Appl. Biol.*, 2013, **162**, 145-173.
43 F. Langa, P. L. Cruz, A. L. Hoz, A. Díaz-Ortiz and E. Díaz-Ortiz, *Contemp. Org. Synth.*, 1997, **4**, 373-386.
44 M. Nüchter, B. Ondruschka, W. Bonrath and A. Gum, *Green Chem.*, 2004, **6**, 128-141.
45 C. O. Kappe, *Chem. Soc. Rev.*, 2008, **37**, 1127-1139.
46 M. D. Bowman, J. L. Holcomb, C. M. Kormos, N. E. Leadbeater and V. A. Williams, *Org. Process Res. Dev.*, 2008, **12**, 41-57.
47 S. Caddick and R. Fitzmaurice, *Tetrahedron*, 2009, **65**, 3325-3355.
48 S. E. López, J. Restrepo, B. Pérez, S. Ortiz and J. Salazar, *Bull. Korean Chem. Soc.*, 2009, **30**, 1628-1630.
49 C. O. Kappe, *Angew. Chem. Int. Ed.*, 2004, **43**, 6250-6284.
50 P. Lidström, J. Tierney, B. Wathey and J. Westman, *Tetrahedron*, 2001, **57**, 9225-9283.
51 L. Y. Fan, Y. H. Shang, X. X. Li, W. J. Hua, *Chin. Chem. Lett.*, 2014, **26**, 77-80.
52 U. R. Pratap, J. R. Mali, D. V. Jawale and R. A. Mane, *Tetrahedron Lett.*, 2009, **50**. 1352-1354.
53 Z. Wang, R. Tang, J. Li, *Chin. J. Chem.*, 2011, **29**, 314-320.
54 K. M. Khan, F. Rahim, S. A. Halim, M. Taha, M. Khan, S. Perveen, Z. Haq, M. A. Mesaik, M. I. Choudhary, *Bioorg. Med. Chem.*, 2011, **19**, 4286-4294.
55 I. H. Segel, 1975. Enzyme kinetics: behavior and analysis of rapid equilibrium and steady-state enzyme systems. John Wiley and Sons, New York.
56 S. Sinha, P. Chattopadhyay, I. Pan, S. Chatterjee, P. Chanda, D. Bandyopadhyay, K. Das, S. K. Sen. *Afr. J. Biotechnol.*, 2009, **8**, 6016–6027.
57 B. S. Londhe, U. R. Pratap, J. R. Mali and R. A. Mane, *Bull. Korean Chem. Soc.*, 2010, **31**, 2329-2332.
58 A. A. Weekes, M. C. Dix, M. C. Bagley and A. A. Westwell, *Synth. Commun.*, 2010, **40**, 3027-3032.
59 N. Park, Y. Heo, M. R. Kumar, Y. Kim, K. H. Song and S. Lee, *Eur. J. Org. Chem.*, 2012, 1984-1993.
60 K. Serdons, T. Verduyckt, D. Vanderghinste, J. Cleynhens, P. Borghgraef, P. Vermaelen, C. Terwinghe, F. Van Leuven, K. Van Laere, H. Kung, G. Bormans and A. Verbruggen, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 602-605.
61 H. Sharghi and O. Asemani, *Synth. Commun.*, 2009, **39**, 860-867.
62 B. George and E. P. Papadopoulos, *J. Org. Chem.*, 1977, **42**, 441-443.
63 P. B. Gorepatil, Y. D. Mane and V. S. Ingle, *Synlett*, 2013, **24**, A-D.
64 T. G. Deligeorgiev, *Dyes Pigments*, 1990, **12**, 243-248.
65 J. S. Baum and T. M. Chen, *US Patent 4556411*, 1985.
66 D. Shi, S. Rong and G. Dou, *Synth. Commun.*, 2010, **40**, 2302-2310.
67 G. H. Sung, I. Lee, B. R. Kim, D. Shin, J. Kim, S. Lee and Y. Yoon, *Tetrahedron*, 2013, **69**, 3530-3535.
68 M. W. Weatherburn, *Analytical Chem.*, 1967, **39**, 971-974.
69 E. Kandeler and H. Gerber, *Biol. Fertil. Soil*, 1988, **6**, 68-72.