Received: 29 March 2018 Revised: 22 May 2018 Accepted article published: 8 June 2018

(wileyonlinelibrary.com) DOI 10.1002/ps.5111

Benzoxazoles as novel herbicidal agents

Diego P Sangi,[a*] Yuri G Meira,[a] Natália M Moreira,[a] Thais A Lopes,[a] Mathias P Leite,[a] Milton E Pereira-Flores[b] and Elson S Alvarenga[c]

Abstract

BACKGROUND: Despite the need to develop new herbicides with different modes of action, due to weed resistance, many important classes of compounds have been studied poorly for this purpose. Benzoxazoles are considered privileged structures because of their biological activities, but their phytotoxic activities have not received a lot of attention until now.

RESULTS: Double vinylic substitution reactions were carried out to furnish four 2-nitromethylbenzoxazoles and one oxazolidine. Benzoxazol-2-ylmethanamine was obtained by reduction of compound 3a. These compounds were evaluated for their phytotoxicity in *Allium cepa* (onion), *Solanum lycopersicum* (tomato), *Cucumis sativus* (cucumber) and *Sorghum bicolor* (sorghum). Comparison with oxazolidine analogue allowed us to understand that the benzoxazolic structure is very important for the herbicidal activity.

CONCLUSION: All the synthesized compounds exhibited biological activity on seed germination. The four 2-nitromethylbenzoxazoles showed phytotoxic activity and the 5-chloro-2-(nitromethyl)benzo[*d*]oxazole (3b) exhibited higher inhibition than the commercial herbicide against all four plant species tested.

Keywords: herbicide; benzoxazoles; synthesis; sorghum; cucumber; onion; tomato

1 INTRODUCTION

Aromatic heterocyclic compounds are known for their wide range of biological activities. Among the heterocyclic classes, the benzoxazole scaffold is a constituent of several natural products and many synthetic drugs (Fig. 1).[1]

Benzoxazole derivatives have been used in medicinal chemistry and many compounds have substantial chemotherapeutic activity, including antiviral,[5,6] antibacterial,[6–9] antifungal[6–9] and anticancer.[6,10] Because of this, benzoxazole derivatives have been considered privileged scaffolds able to bind multiple targets and provide new prototypes for drug design.[11,12]

Benzoxazoles substituted at position 2 are usually synthesized by coupling carboxylic acid derivatives and 2-aminophenols, frequently with the aid of catalysts, acidic conditions and high temperature.[13–15] By contrast, many methods use condensation between a 2-aminophenol and an aldehyde, with subsequent oxidation.[16,17] In a search for environmentally friendly, milder conditions and easier procedures, we recently developed a synthesis using a double vinylic substitution of 1,1-bis-methylsulfanyl-2-nitroethylene (**1**) and a 2-aminophenol (**2**).[18]

Despite their wide use in medicinal chemistry, low levels of cytotoxicity and the facile synthesis of benzoxazole derivatives, these compounds have not received much attention regarding their potential use in agrochemical research.

Aryloxyphenoxypropionic acid derivatives belong to a class of herbicides containing benzoxazolyl substituents, as in fenoxiprop and metamifop. However, these substituents are not crucial for their activity, as shown by haloxyfop and fluazifop which maintain the same level of activity without the benzoxazolyl group (Fig. 2).[19]

Dietrich *et al.* synthesized and evaluated the herbicidal activity of a library of 300 benzoxazolylamino-1,3,5-triazines.[20] They investigated the effect of a benzoxazolyl group as a substituent in the 1,3,5-triazines, a known class of herbicide. This work was inspired by a patent published by Giencke *et al.* that showed the herbicidal activity of 2,4-diamino-1,3,5-triazines, including some compounds containing the benzoxazolyl group.[21]

More recently, Fukuda *et al.* published a patent presenting the herbicidal activity of 2-substituted benzoxazoles containing aryl and heteroaryl substituents, but the mechanism of action was not determined.[22]

In this study, we synthesized a series of 2-substituted benzoxazoles (**3**) and evaluated their phytotoxic activity on several crops to assess their structure–activity relationships.

2 MATERIALS AND METHODS

2.1 General procedures

Reagents and solvents were used without any prior purification. The microwave-assisted synthesis was conducted in an Anton Paar Monowave 200, a single-mode oven with infrared sensors to

---

[*] *Correspondence to: DP Sangi, Chemistry Department, Universidade Federal Fluminense, Volta Redonda, RJ, Brazil. E-mail: dpsangi@id.uff.br*

[a] *Chemistry Department, Universidade Federal Fluminense, Volta Redonda, Brazil*

[b] *Department of Agricultural and Environmental Engineering, Universidade Federal de Viçosa, Brazil*

[c] *Chemistry Department, Universidade Federal de Viçosa, Viçosa, Brazil*

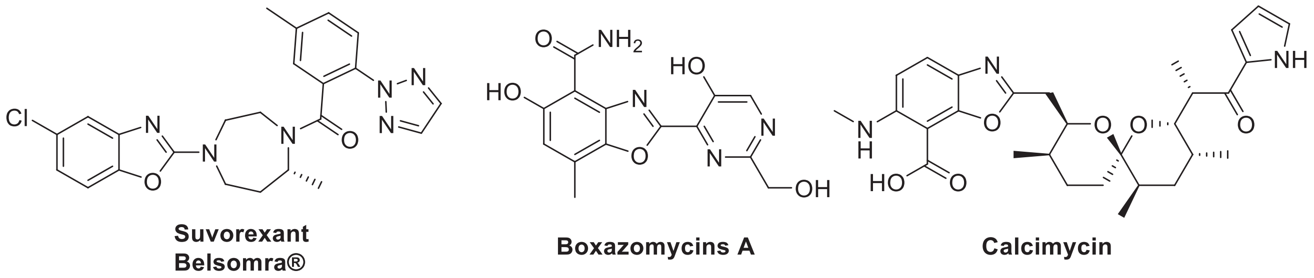


Figure 1. Examples of drugs and natural products containing a benzoxazole heterocycle: suvorexant,[2] boxazomycins A[3] and calcimycin.[4]

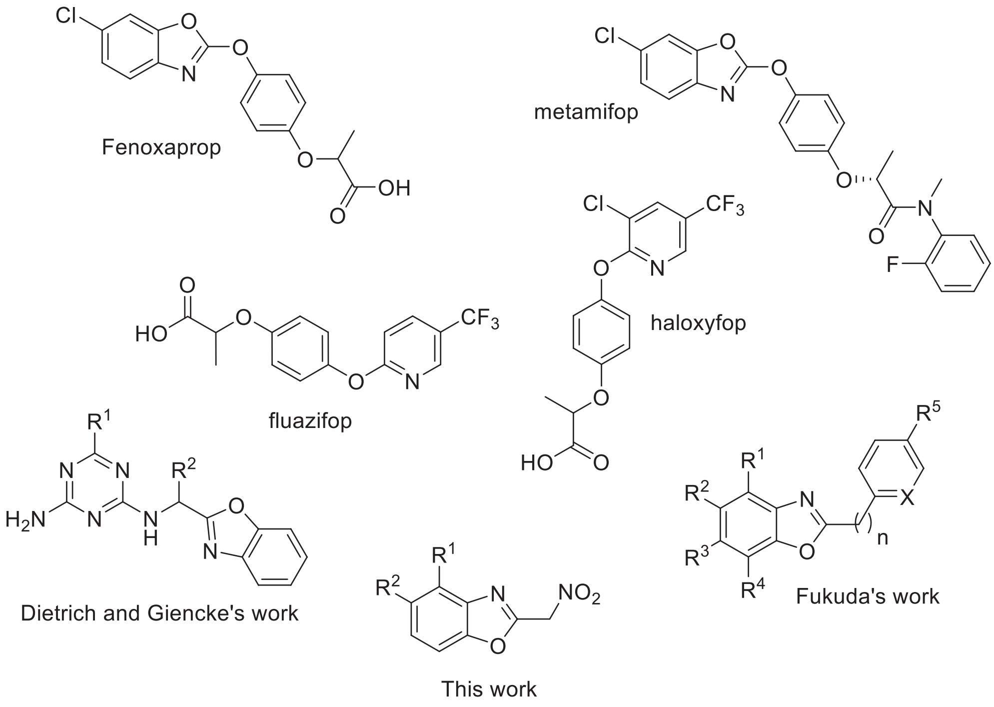


Figure 2. Commercial herbicides and benzoxazoles derivatives.

control the potency and afford the selected temperature. All the compounds tested were purified via flash column chromatography and their purity was assessed by gas chromatography (GC) and nuclear magnetic resonance (NMR). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker ARX-400 (400 and 100 MHz, respectively). Mass spectra were recorded on a Shimadzu GCMS-QP5000.

2.2 Synthetic procedures

Nitroethylene **1** (165 mg 1.0 mmol), 2-aminoalcohols (1.0 mmol) and ethanol (4 mL) were placed in a glass tube, sealed and irradiated for 60 min in an Anton Paar Monowave 200 oven at 110 °C. The obtained 2-nitromethylbenzoxazoles **3** were purified by flash column chromatography employing dichloromethane as the eluent.

2-(Nitromethyl)benzo[*d*]oxazole (**3a**): ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.83 (s, 2H), 7.42–7.50 (m, 2H), 7.61–7.64 (m, 1H), 7.82–7.85 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 71.67, 111.25, 121.10, 125.32, 126.86, 140.66, 151.39, 153.98. GC-MS (70 eV) *m/z* (%): 178 (M^+, 2), 132 (100), 104 (25), 77 (35).

5-Chloro-2-(nitromethyl)benzo[*d*]oxazole (**3b**): ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.81 (s, 2H), 7.44 (dd, 1H, *J* 8.49, 2.12) 7.54 (dd, 1H, *J* 8.49), 7.80 (d, 1H, *J* 2.12). ^{13}C NMR (100 MHz, $CDCl_3$): δ 71.48, 112.07, 121.04, 127.34, 130.97, 141.67, 149.90, 155.26. GC-MS (70 eV) *m/z* (%): 212 (M^+, 5), 166 (100), 138 (25), 102 (45), 63 (40).

5-Methyl-2-(nitromethyl)benzo[*d*]oxazole (**3c**): ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.50 (s, 3H), 5.78 (s, 2H), 7.25–7.29 (m, 1H), 7.47 (d, 1H, *J* 8.37), 7.57–7.60 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.48, 71.70, 110.58, 120.82, 128.06, 135.32, 140.84, 153.95, 153.97.

4-Methyl-2-(nitromethyl)benzo[*d*]oxazole (**3d**): ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.64 (s, 3H), 5.80 (s, 2H), 7.21 (d, 1H, *J* 7.51), 7.38–7.34 (dd, 1H, *J* 7.51, 8.07), 7.41 (d, 1H, *J* 8.07). ^{13}C NMR (100 MHz, $CDCl_3$): δ 16.42, 71.69, 108.46, 125.74, 126.55, 131.71, 139.99, 151.19, 153.15. GC-MS (70 eV) *m/z* (%): 192 (M^+, 3), 146 (100), 117 (18), 91 (32).

2-(Nitromethylene)oxazolidine (**5**): ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.14–3.56 (m, 4H), 6.53 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 44.71, 59.92, 97.69, 156.75.

A suspension of 2-(nitromethyl)benzo[*d*]oxazole **3a** (178.15 mg, 1.0 mmol), palladium on carbon 10% (17.82 mg, 10 wt% of the substrate) as a catalyst, in methanol (10 mL) was stirred under a hydrogen atmosphere (1 atm) at 25 °C. The progress of the reactions was followed by silica-gel thin-layer chromatography. After 2 h the reaction mixture was filtered and concentrated under vacuum. The crude product was purified by silica-gel column chromatography using dichloromethane as the eluent.[23]

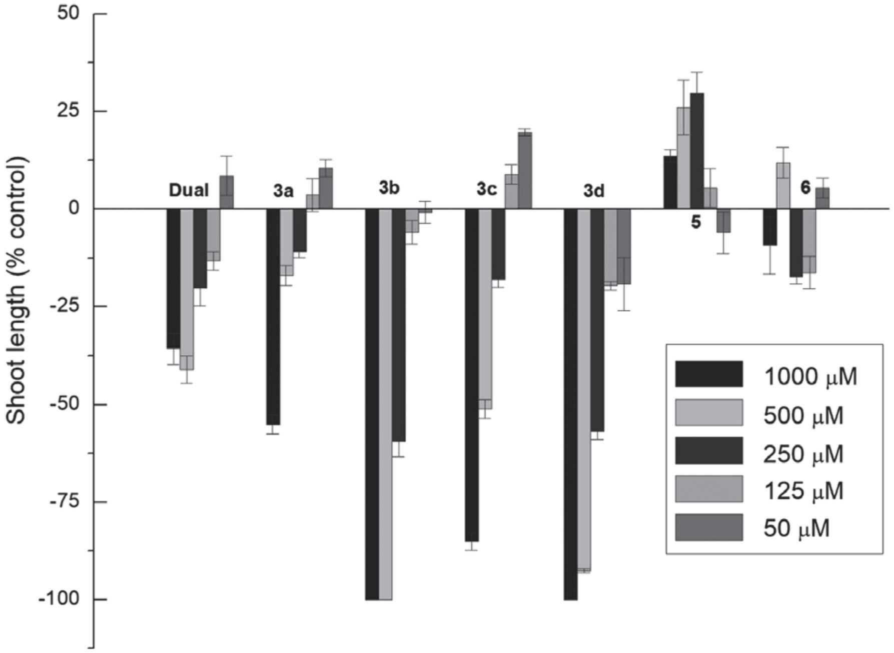


Figure 3. Shoot length of tomato compared with *S*-metolachlor. Values are expressed as percentage difference from the control.

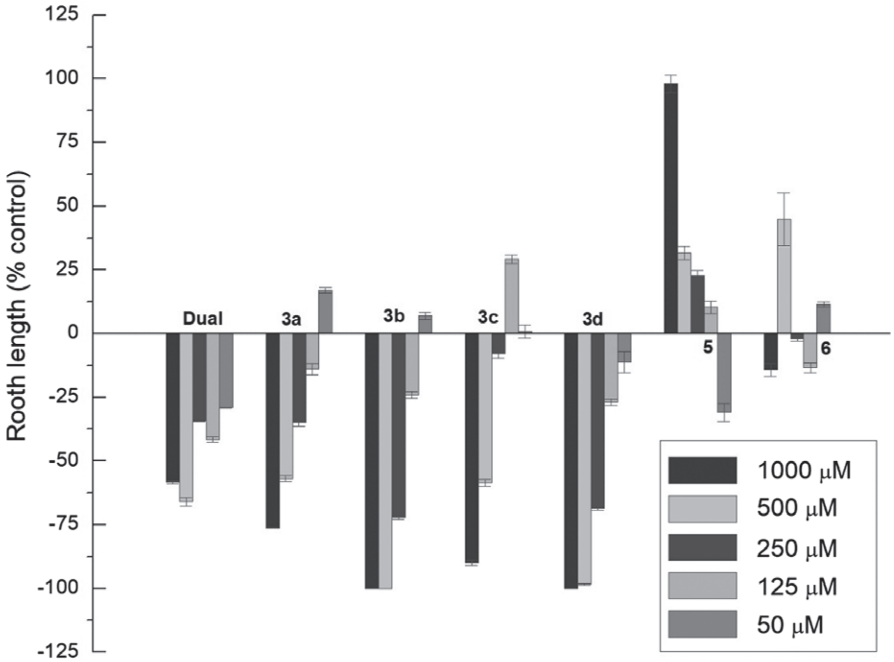


Figure 4. Root length of tomato compared with *S*-metolachlor. Values are expressed as percentage difference from the control.

Benzoxazol-2-ylmethanamine (**6**): ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.39 (s, 2H), 7.30–7.35 (m, 2H), 7.50–7.52 (m, 1H), 7.68–7.72 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 51.62, 111.10, 120.43, 124.96, 125.59, 141.22, 151.21, 164.14.

3 BIOASSAYS[24]

The general procedure was as follows. Twenty seeds of *Allium cepa* (onion) and *Solanum lycopersicum* (tomato) were placed in a Petri dish (9 cm in diameter), with 5 mL of test solution, three replicates of each concentration, and maintained for 9 and 7 days in the dark at 25 °C. Seeds of *Cucumis sativus* (cucumber) and *Sorghum bicolor* (sorghum) are bigger than the others, so the roots and shoots are very bulky. Therefore, their development was hindered when the bioassay was carried out in 9 cm Petri dishes. Therefore, 20 seeds were placed in each 15-cm diameter petri dish with 8 mL of test solution for 6 days in the dark, at 25 °C.

Compounds **3a**–**d**, **5**, **6** and *S*-metolachlor were weighed, dissolved in dimethyl sulfoxide (DMSO) and diluted with distilled water to prepare 30 mL of an aqueous solution containing DMSO 0.3% v/v. Half of this solution was used in the bioassays, and the other 15 mL was diluted with 15 mL of distilled water containing

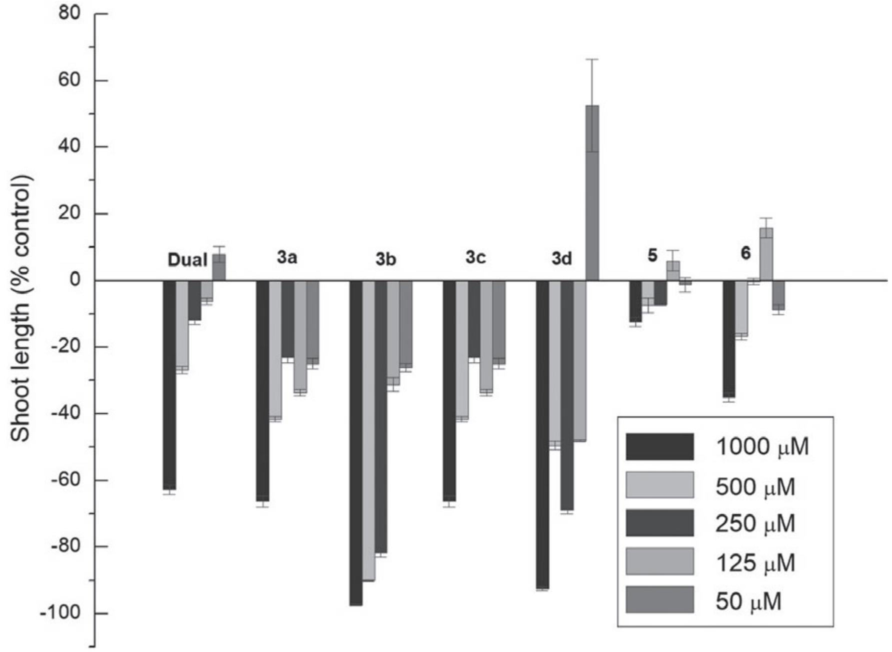


Figure 5. Shoot length of onion compared with *S*-metolachlor. Values are expressed as percentage difference from the control.

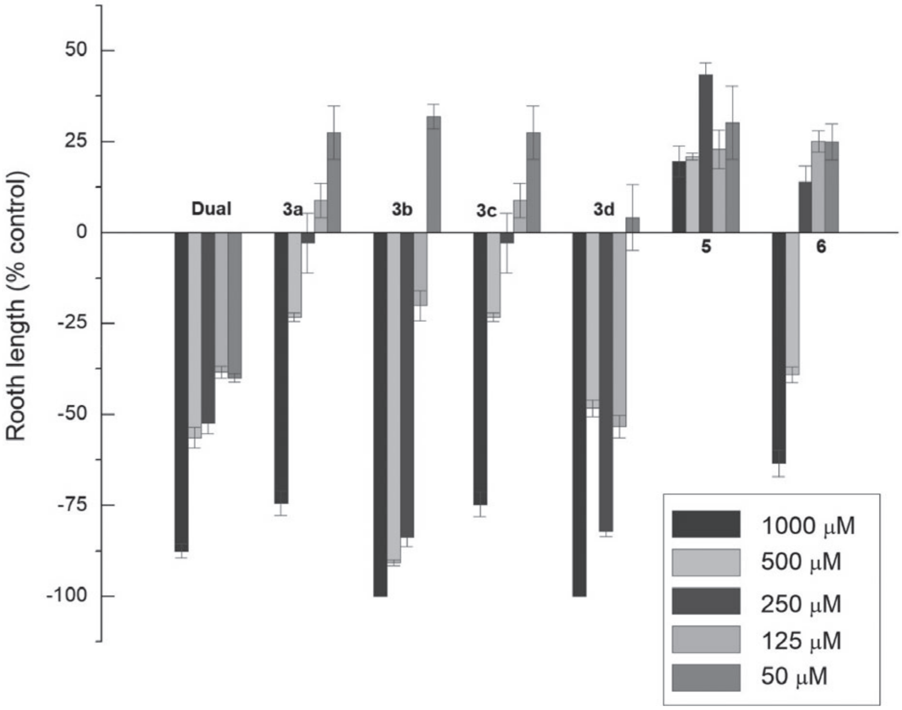


Figure 6. Root length of onion compared with *S*-metolachlor. Values are expressed as percentage difference from the control.

0.3% DMSO v/v to prepare the less-concentrated solution. Aqueous DMSO 0.3% v/v was used as a negative control and the pre-emergence commercial herbicide Dual (*S*-metolachlor) was used as a positive control. All bioassays were carried out with the solutions at concentrations of 1000, 500, 250, 125 and 50 μM. After the germination period, the seeds were digitally photographed and measured.

The results are presented as percentage differences from the control in bar graphs with standard deviation error bars (Figs 3–10). Thus, zero represents the control, positive values represent stimulation of the studied parameters and negative values represent inhibition.

4 RESULTS AND DISCUSSION

4.1 Synthesis

Using a procedure described in the literature,[18] we prepared the 2-nitromethylbenzoxazoles **3a–d**, employing the double vinylic substitution assisted by microwave irradiation between 1,1-bis-methylsulfanyl-2-nitroethylene **1** and 2-aminophenols

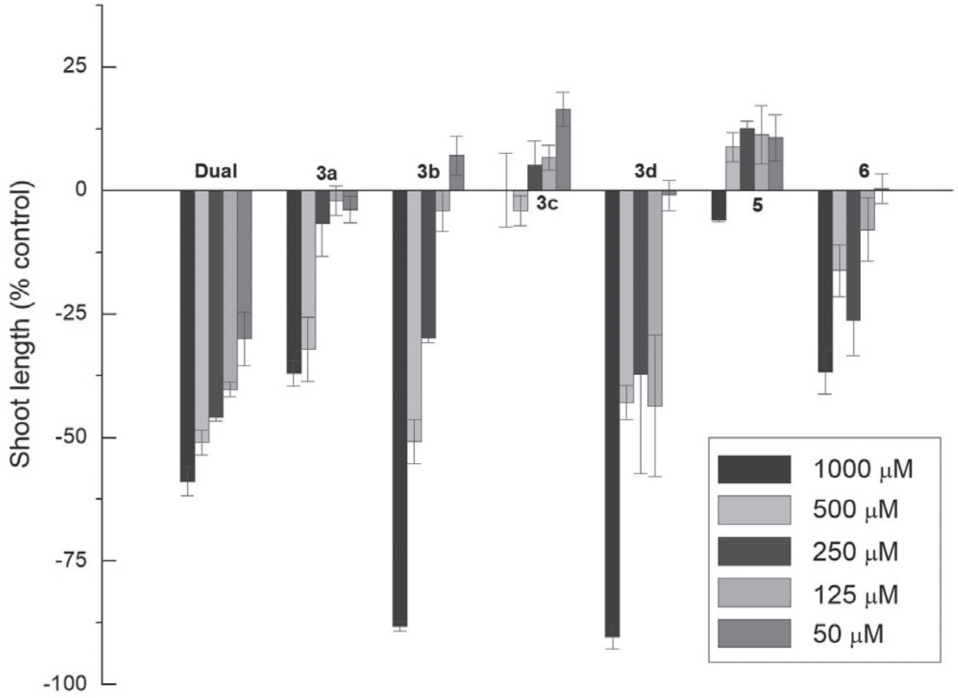


Figure 7. Shoot length of cucumber compared with *S*-metolachlor. Values are expressed as percentage difference from the control.

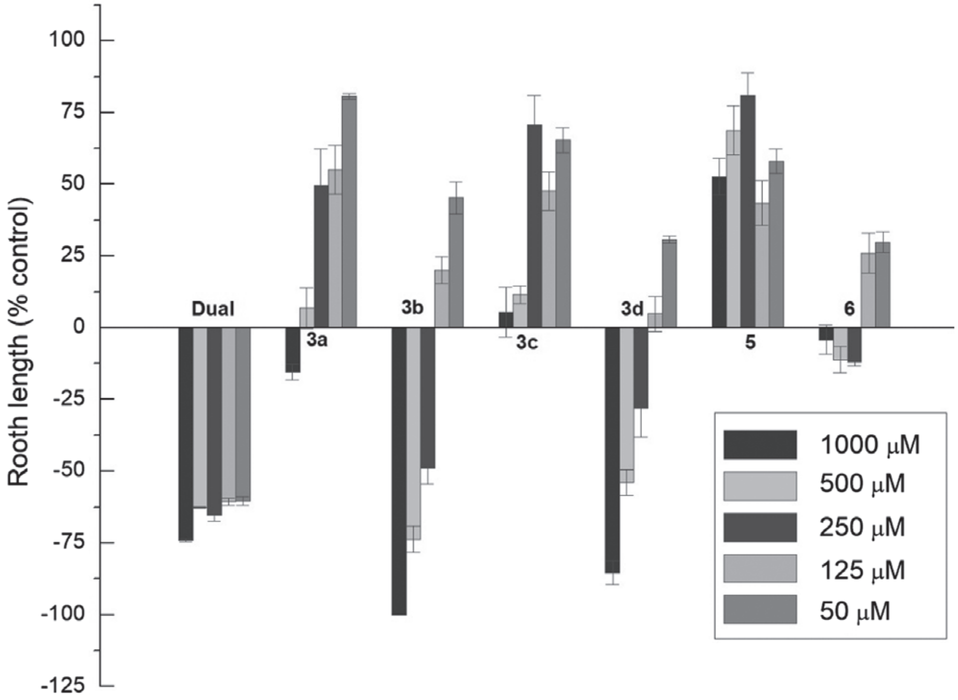


Figure 8. Root length of cucumber compared with *S*-metolachlor. Values are expressed as percentage difference from the control.

2. Compounds **3a**–**3d** were purified using silica-gel column chromatography and were easily identified by the presence of the signal corresponding to aromatic hydrogens and lack of methylsulfanyl hydrogens in the ^{1}H NMR spectra (Scheme 1).[18]

Searching for the contribution of the benzenoid portion in the heterocyclic compound, we tested oxazolidine **5**. The 2-nitromethylene-oxazolidine **5** was obtained via the same synthetic method, using 2-aminoethanol as a nucleophile for the double vinylic substitution. After filtration, the formation of oxazolidine **5** was detected by the presence of the signal of the alkenyl hydrogen at δ 6.53 ppm, and absence of methylsulfanyl hydrogens in the ^{1}H NMR (Scheme 2).

The nitro group in the benzoxazole **3a** was reduced under a hydrogen atmosphere catalyzed with palladium on carbon to produce the benzoxazol-2-ylmethanamine **6**. The carbon and hydrogens of the methylene group of compound **6** were displaced to a

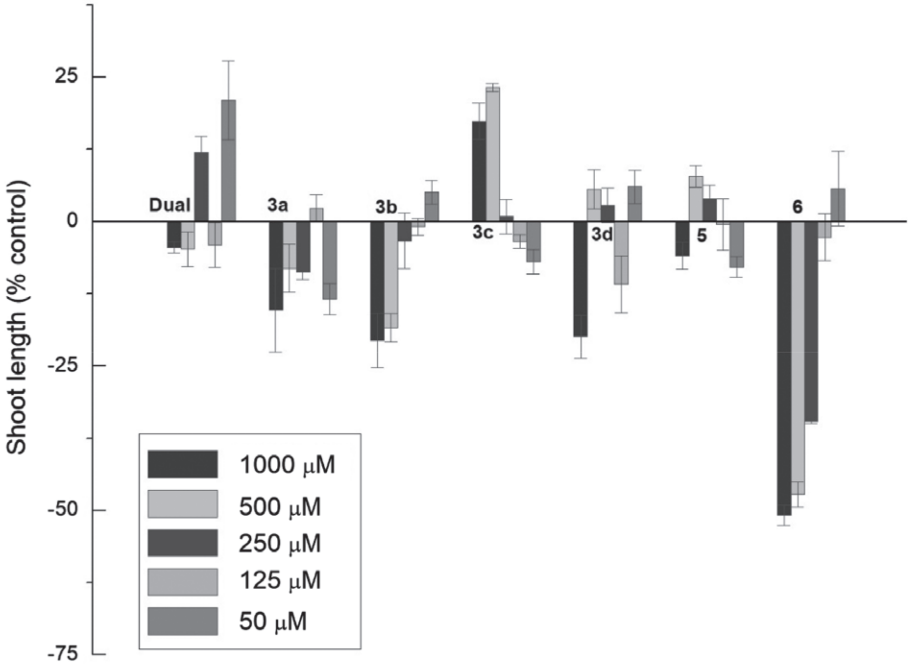


Figure 9. Shoot length of sorghum compared with *S*-metolachlor. Values are expressed as percentage difference from the control.

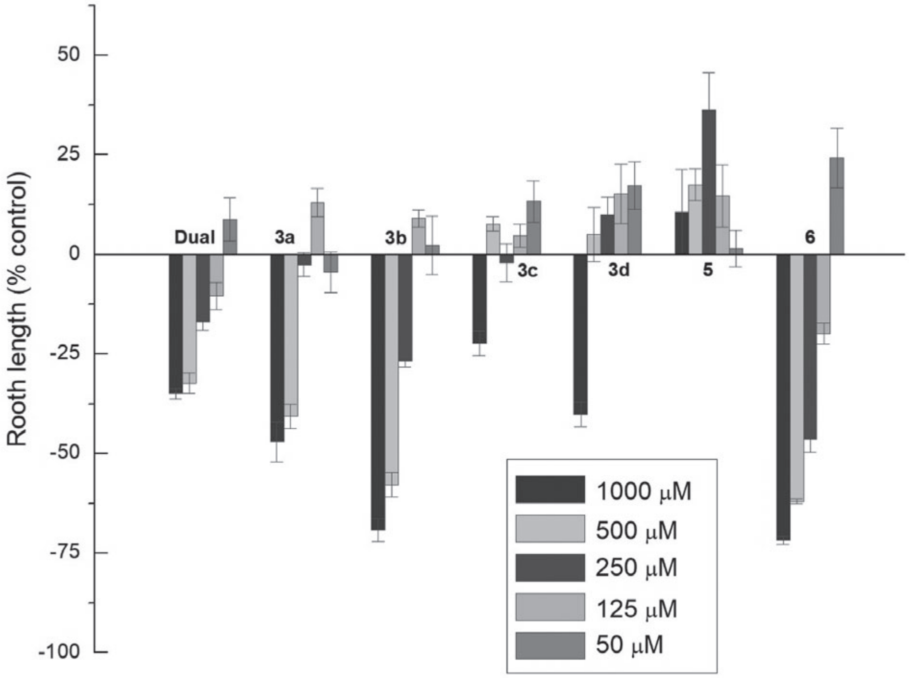


Figure 10. Root length of sorghum compared with *S*-metolachlor. Values are expressed as percentage difference from the control.

higher magnetic field in the ^{13}C and ^{1}H NMR spectra, respectively, when compared with the starting material **3a**, which is a key evidence for the conversion (Scheme 3).

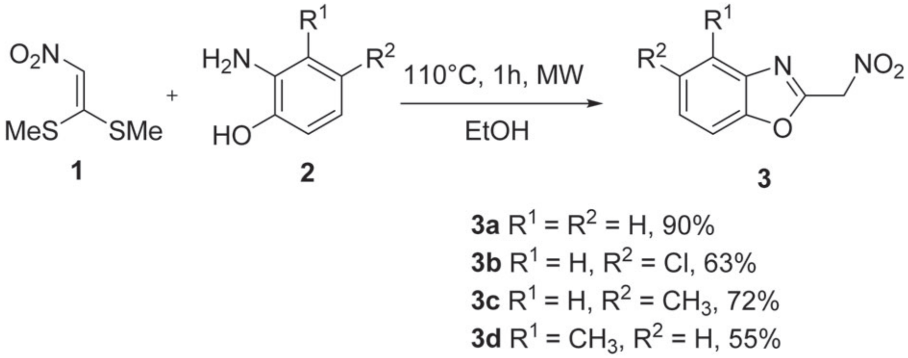
4.2 Biological activity

4.2.1 Solanum lycopersicum *(Figs 3 and 4)*

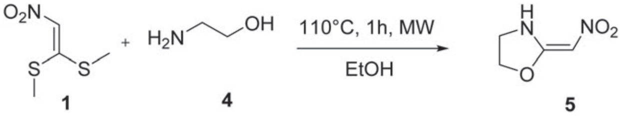
All tested 2-nitromethylbenzoxazoles (**3a**–**3d**) showed high phytotoxicity, superior to *S*-metolachlor. The most active compound was **3b** which inhibited 100% of aerial and root growth at 1000 and 500 μM. The presence of the nitro group seems to be important for the phytotoxic activity, because compound **6** was less active than compounds **3a**–**3d**. Oxazolidine **5** showed a totally different activity pattern. Surprisingly, it stimulated aerial and root growth. This allowed us to conclude that the benzenoid ring is very important for the phytotoxic activity in tomato seeds.

4.2.2 Allium cepa *(Figs 5 and 6)*

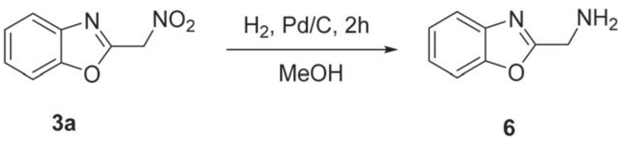
Compounds **3a** and **3c** exhibited similar activities to *S*-metolachlor, but compounds **3b** and **3d** showed higher levels of phytotoxicity, both inhibited 100% of the aerial and root growth at 1000 μM. Once



Scheme 1. Double vinylic substitution to synthesize benzoxazole derivatives.



Scheme 2. Synthesis of 2-nitromethylene-oxazolidine.



Scheme 3. Synthesis of benzoxazol-2-ylmethanamine.

again, compound **6** exhibited lower activity than the nitromethyl benzoxazoles and *S*-metolachlor, indicating the importance of the nitro group for the activity and the oxazolidine compound **5** presented a different activity pattern, stimulating the root growth.

4.2.3 Cucumis sativus *(Figs 7 and 8)*

Compounds **3a** and **3c** presented lower activity than *S*-metolachlor. However, compounds **3b** and **3d** showed higher phytotoxic activity than *S*-metolachlor in shoot and root assays. Compound **3b** was the most active inhibiting 100% of the roots and almost 90% of shoot growth at 1000 μM, whereas the reduced benzoxazole **6** did not show relevant inhibition. However, oxazolidine **5** presented a pronounced stimulation of the growth of the roots.

4.2.4 Sorghum bicolor *(Figs 9 and 10)*

Compounds **3a** and **3b** showed higher levels of inhibition than *S*-metolachlor, but surprisingly the most phytotoxic compound was the derivative **6**. Compound **6** showed much more inhibition of roots and shoots than the commercial herbicide. Once again oxazolidine **5** did not show phytotoxic activity.

5 CONCLUSIONS

Employing the double vinylic substitution in 1,1-bismethylsulfanyl-2-nitroethylene, four 2-nitromethylbenzoxazole derivatives (**3a**–**d**) were prepared. These compounds were evaluated for their potential phytotoxic activity in *Allium cepa*, *Solanum lycopersicum*, *Cucumis sativus* and *Sorghum bicolor*.

The structure–activity relationship was evaluated by testing the oxazolidine **5**, which was prepared by double vinylic substitution. The nitro group of compound **3a** was hydrogenated to afford the benzoxazole **6**.

All compounds were found to exhibit activity in seed germination. Compound **3b** proved to be more phytotoxic than *S*-metolachlor (commercial herbicide Dual) for all four species tested, being able to inhibit 100% germination in *Allium cepa*, *Solanum lycopersicum*, and *Cucumis sativus*. Compound **6** was the most active against *Sorghum bicolor*, being more active than *S*-metolachlor. By contrast, compound 5 exhibited stimulating activity for all species tested.

We can conclude that benzoxazoles represent a class of compounds with great potential for development of novel herbicides.

ACKNOWLEDGEMENTS

The authors would like to thanks the Brazilian Agencies CNPq, FAPERJ, FAPEMIG, RQ-MG, and PROPPI-UFF for financial support.

REFERENCES

1 Demmer CS and Bunch L, Benzoxazoles and oxazolopyridines in medicinal chemistry studies. *Eur J Med Chem* **97**:778–785 (2015).
2 Cox CD, Breslin MJ, Whitman DB, Schreier JD, McGaughey GB, Bogusky MJ *et al.*, Discovery of the dual orexin receptor antagonist [(7*R*)-4-(5-chloro-1,3-benzoxazol-2-yl)-7-methyl-1,4-diazepan-1-yl] [5-methyl-2-(2H-1,2,3-triazol-2-yl)phenyl]methanone (MK-4305) for the treatment of insomnia. *J Med Chem* **53**:5320–5332 (2010).
3 Kusumi T, Ooi T, Walchli MR and Kakisawa H, Structure of the novel antibiotics boxazomycins A, B, and C. *J Am Chem Soc* **110**:2954–2958 (1988).
4 Chaney MO, Demarco PV, Jones ND and Occolowitz JL, The structure of A23187, a divalent cation ionophore. *J Am Chem Soc* **96**:1932–1933 (1974).
5 Jonckers THM, Rouan MC, Haché G, Schepens W, Hallenberger S, Baumeister J *et al.*, Benzoxazole and benzothiazole amides as novel pharmacokinetic enhancers of HIV protease inhibitors. *Bioorg Med Chem Lett* **22**:4998–5002 (2012).
6 Rida SM, Ashour FA, El-Hawash SAM, ElSemary MM, Badr MH and Shalaby MA, Synthesis of some novel benzoxazole derivatives as anticancer, anti-HIV-1 and antimicrobial agents. *Eur J Med Chem* **40**:949–959 (2005).
7 Seenaiah D, Reddy PR, Redy GM, Pagmaja A, Padmavathi V and Krishna NS, Synthesis, antimicrobial and cytotoxic activities of pyrimidinyl benzoxazole, benzothiazole and benzimidazole. *Eur J Med Chem* **77**:1–7 (2014).
8 Ertan T, Yildiz I, Tekiner-Gulbas B, Bolelli K, Temiz-Arpaci O, Yalcin I *et al.*, Synthesis, biological evaluation and 2D-QSAR analysis of benzoxazoles as antimicrobial agents. *Eur J Med Chem* **44**:501–510 (2009).
9 Zhang W, Liu J, Macho JM, Jiang X, Xie D, Jiang F *et al.*, Design, synthesis and antimicrobial evaluation of novel benzoxazole derivatives. *Eur J Med Chem* **126**:7–14 (2017).

10 McKee ML and Kerwin SM, Synthesis, metal ion binding, and biological evaluation of new anticancer 2-(20-hydroxyphenyl)benzoxazole analogs of UK-1. *Bioorg Med Chem* **16**:1775–1783 (2008).
11 Welsch ME, Snyder SA and Stockwell BR, Privileged scaffolds for library design and drug discovery. *Curr Opin Chem Biol* **14**:347–361 (2010).
12 Evans BE, Rittle KE, Bock MG, DiPardo RM, Freidinger RM, Whitter WL *et al.*, Methods for drug discovery: development of potent, selective, orally effective cholecystokinin antagonists. *J Med Chem* **31**:2235–2246 (1988).
13 Mary YS, Al-Shehri MM, Jalaja K, Al-Omary FAM, El-Emam AA, Panicker CY *et al.*, Synthesis, vibrational spectroscopic investigations, molecular docking, antibacterial studies and molecular dynamics study of 5-[(4-nitrophenyl)acetamido]-2-(4-*tert*-butylphenyl)benzoxazole. *J Mol Struct* **1133**:557–573 (2017).
14 Azizian J, Torabi P and Noei J, Synthesis of benzimidazoles and benzoxazoles using TiCl3OTf in ethanol at room temperature. *Tetrahedron Lett* **57**:185–188 (2016).
15 Wang Y, Wu C, Nie S, Xu D, Yu M and Yao X, Ligand-promoted, copper nanoparticles catalyzed one-pot synthesis of substituted benzoxazoles from 2-bromoanilines and acyl chlorides. *Tetrahedron Lett* **56**:6827–6832 (2015).
16 Ziarati A, Sobhani-Nasab A, Rahimi-Nasrabadi M, Ganjali MR and Badiei A, Sonication method synergism with rare earth based nanocatalyst: preparation of $NiFe_{2}\text{-}_{x}Eu_{x}O_{4}$ nanostructures and its catalytic applications for the synthesis of benzimidazoles, benzoxazoles, and benzothiazoles under ultrasonic irradiation. *J Rare Earths* **35**:374–381 (2017).
17 Padalkar VS, Borse BN, Gupta VD, Phatangare KR, Patil VS, Umape PG *et al.*, Synthesis and antimicrobial activity of novel 2-substituted benzimidazole, benzoxazole and benzothiazole derivatives. *Arab J Chem* **9**:S1125–S1130 (2016).
18 Sangi DP, Monteiro JL, Vanzolini KL, Cass QB, Paixão MW and Corrêa AG, Microwave-assisted synthesis of N-heterocycles and their evaluation using an acetylcholinesterase immobilized capillary reactor. *J Braz Chem Soc* **25**:897–899 (2014).
19 Kobek K, Focke M and Botanisches KL, Fatty-acid biosynthesis and acetyl-CoA carboxylase as a target of diclofop, fenoxaprop and other aryloxy-phenoxy-propionic acid herbicides. *Zeitschrift für Naturforschung C* **43**:47–54 (1988).
20 Dietrich H, Giencke W and Klein R, The design and synthesis of a herbicide targeted library of *N*-[1-(1,3-benzoxazol-2-yl)alkyl]-6-alkyl-1,3,5-triazine-2,4-diamines. *Comb Chem High Throughput Screen* **8**:623–629 (2005).
21 Glencke W, Minn K, Willms L, Auler T, Bieringer H, Rosinger C, 2,4-Diamino-1,3,5-triazines, their preparation, and their use as herbicides and plant growth regulators. US 6884758 B2 (2005).
22 Fukuda S, Nakamura A, Shimizu M, Okada T, Oohida S, Asahara T, Benzoxazole compounds, process for producing the same and herbicides. US 7402548 B2 (2008).
23 Mori A, Miyakawa Y, Ohashi E, Haga T, Maegawa T and Sajiki H, Pd/C-catalyzed Chemoselective hydrogenation in the presence of diphenylsulfide. *Org Lett* **8**:3279–3281 (2006).
24 Moraes FC, Alvarenga ES, Amorim KB, Demuner AJ and Pereira-Flores ME, Novel platensimycin derivatives with herbicidal activity. *Pest Manag Sci* **72**:580–584 (2015).