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NEW ANTIFUNGAL AGENTS: SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITY OF SOME MANNICH BASES DERIVED FROM 2-MERCAPTOBENZOTHIAZOLE

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Abstract– A series of new *3-[(amino)methyl]-1,3-benzothiazole-2-thione* **4(a-l)** were synthesized by Mannich reaction from formaldehyde, aliphatic or aromatic amines and 2-mercaptobenzothiazole. All synthesized compounds were in good agreement with elemental and spectral data (^{1}H NMR and mass spectroscopy) and were evaluated for *in vitro* antifungal activity by agar well diffusion method. Fluconazole was included in the assays as a commercially available reference compound. Four fungal strains were used to perform the study: *Cladosporium cladosporioides*, *Aspergillus niger* ATCC 16404, *Botrytis* sp. and *Rhizopus* sp. Compounds **4a-c**, **4e-g**, **4i** and **4l** were found to be active against the four moulds mentioned, due to the presence of inhibitory halo. **4h** was active in case of *C. cladosporioides*. Compounds **4d**, **4j** and **4k** did not show inhibition against fungi used for the test. Fluconazole showed biological activity against *Aspergillus niger* ATCC 16404 and *Botrytis* sp. Therefore, 8 of the 12 synthesized substances would be considered as promising products to the treatment of fungal diseases.

INTRODUCTION

Antimicrobial resistance (AMR) represents one of the greatest threats to global public health. Resistance becomes a public health problem when resistant strains compromise the effectiveness of prescribed antibiotic therapy. The resistant microorganisms make up a large reservoir of genes that can potentially transfer resistance to human, animal and environmental pathogens. Using the whole genome sequencing, experts can identify resistant genes in bacteria, rather than current phenotypic methods that test bacteria for resistance to specific antibiotics. This, does not only have the potential to predict AMR more efficiently, it also generates a wealth of data that can be used for other epidemiological studies and analyzes. Also, from genomics it is feasible to identify new pharmacological targets and contribute to the design of new antimicrobial agents with a defined structure and a more efficient mode of action (Walsh *et al.*, 2000; Ritter and Wong, 2001).

As the incidence and prevalence of invasive fungal infections has increased dramatically in the last 20 years, the development of new, more selective and less toxic antifungal drugs has become imperative (Maertens and Boogaerts, 2005; Datry and Bart-Delabesse, 2006). In this direction, the scientific community has explored numerous selection methods to evaluate different natural or synthetic drugs. In this sense, 2-Mercaptobenzothiazole (2-MBT) constitutes an important pharmacophore, possessing several pharmacological functions. 2-MBT and its

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derivatives have shown to have biological activity as potent antimicrobials and antifungals agents (Quiroga *et al.*, 2002; Kok *et al.*, 2007; Grover and Moore, 1962), neurotransmission inhibitors (Benavides *et al.*, 1985; Mizoule *et al.*, 1985) and as antitumor agents (Leong *et al.*, 2004; Yldiz-Oren *et al.*, 2004; Lockhart *et al.*, 2005). The molecule of 2-MBT contain extended π-delocalized systems which are capable of binding to complex molecules such as Deoxyribonucleic Acid (DNA) via π–π interactions and consequently exhibit interesting biological properties, for example, for the treatments of cancer and infectious diseases caused by certain microorganisms such as bacteria, fungi and parasites (Triphati and Mishra, 2007; Pavlovic *et al.*, 2007; Susithra *et al.*, 2022).

The present study was undertaken in order to synthesize and to explore the biological activity of some new compounds having this heterocyclic ring. Therefore, the present work reports the synthesis of some Mannich´s bases of 2-MBT and their *in vitro* antifungal activity as a part of our program aimed at the development of new heterocyclic compounds with potential antifungal activities.

MATERIALS AND METHODS

General materials

All the chemicals and reagents used in synthetic procedures were of reagent grade and used without further purification. These were used as received from Sigma-Aldrich Co., Inc., Saint Louis, USA. The purity of the synthesized compounds was ascertained by Thin Layer Chromatography (TLC) on silica gel GF254 with mixtures of hexane/ethyl acetate (3:1) as solvent systems and using *p*-anisaldehyde as detecting agent. Melting points were determined on a Büchi 510 micro melting point determination apparatus in open capillary tubes and are uncorrected. High-resolution mass spectrometry (HRMS) data were obtained on a Bruker micro QTOF-Q11 mass spectrometer equipped with an electrospray ionization (ESI). Proton ^{1}H NMR spectra were recorded on Bruker Avance Ultra Shield Spectrometer (Bruker, 300 MHz, Russ, Germany) using Chloroform-*d* ($CDCl_3$) and Dimethyl sulfoxide-*d6* (DMSO-*d6*) as solvents and tetramethyl silane (TMS) as an internal standard. Chemical shift value is expressed in delta (δ) parts per million (ppm). Antimicrobial tests were done using potato dextrose agar (PDA) and these were purchased from Britania Lab, Argentina. Microbial strains employed were *Cladosporium cladosporioides*, *Aspergillus niger* ATCC 16404, *Botrytis* sp. and *Rhizopus sp.*, all from the collection of the laboratory of IDIC-UCP Goya, Corrientes, Argentina. Fluconazole (Vannier) was used as a commercially available reference compound.

Synthetic procedures

General procedure for the synthesis of 3-[(amino)methyl]-1,3-benzothiazole-2-thione compounds 4(a-l): In a round-bottomed flask, aqueous formaldehyde (**1**) (5 ml of 37% solution; 0.05 mole) was added dropwise to aliphatic or aromatic amines **2(a-l)** (0.05 mole) under stirring at 30 °C (to aliphatic amines at 5 °C). After formation of a solid white precipitate and with vigorous mixing 2-MBT (**3**) (8.2 g; 0.05 mole) diluted in acetone (2 mL) was added in small amounts under continuous stirring. Behind 10 minutes, the mixture was carefully heated at 55 °C whereas obtained a yellow solution. After standing for 10 min the solution was cooled to 5 °C and diluted with water (60 ml) to obtain needles of product crystallized. Finally, the crystals were filtered and purified by crystallization from ethanol (Figure 1).

By adopting this procedure, 12 compounds were synthesized. Synthetic pathway for preparation of title compounds is shown in Figure 1. The aliphatic and aromatic amines **2(a-l)**, *pka* value of amines and yields for **4(a-l)** products are summarized in Table 1.

Spectral data of synthesized compounds

3-(morpholin-4-ylmethyl)-1,3-benzothiazole-2-thione (**4a**): White solid (25%). Mp = 148-149 °C (Lit. 149-150). ^{1}H NMR [$CDCl_3$, 300MHz]: δ = 2.63 (t, 4H, CH_2), 3.69 (t, 4H, CH_2), 4.91 (s, 2H, CH_2), 7.33-7.42

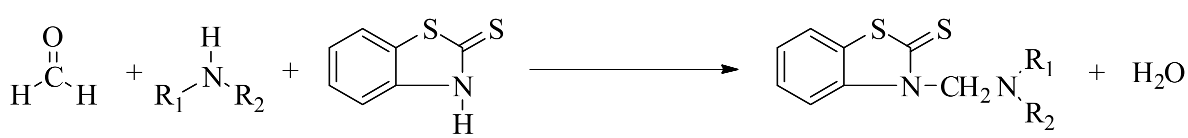


Fig. 1. Synthesis of *3-[(amino)methyl]-1,3-benzothiazole-2-thione* **4(a-l)**

Table 1. Amines, *pKa* values of amines **2(a-l)** and yields for **4(a-l)** compounds

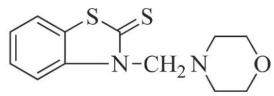
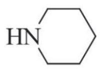
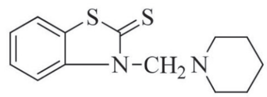
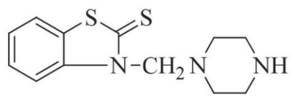
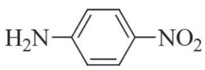
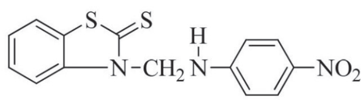
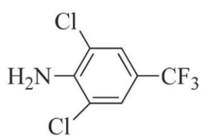
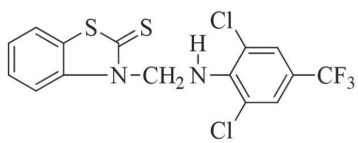
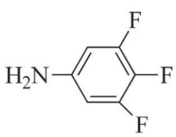
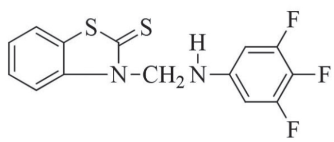
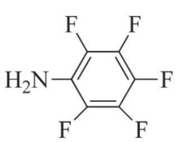
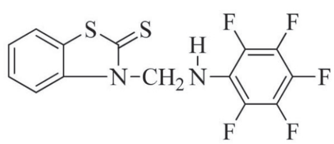
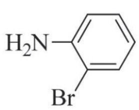
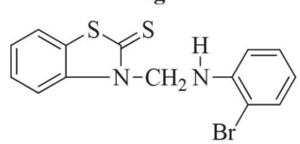
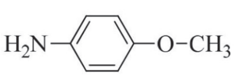
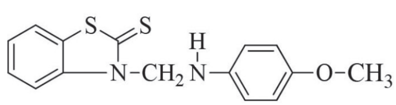
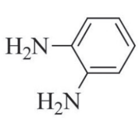
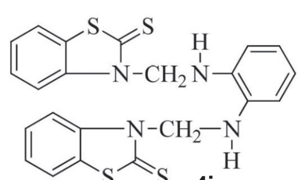
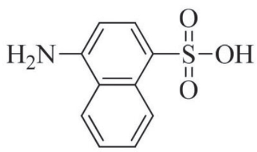
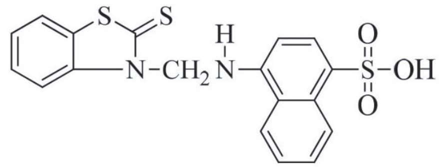
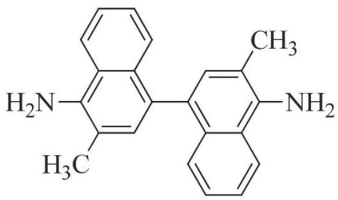
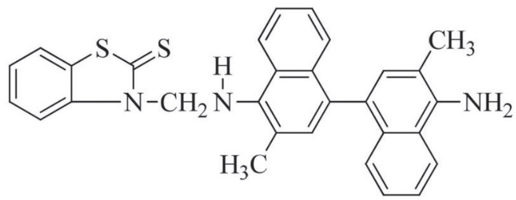
Entry	Amines	*pKa* [a]	Products	Yields (%) [b]
1	 **2a**	8.49	 **4a**	25
2	 **2b**	2.90	 **4b**	50
3	 **2c**	9.73	 **4c**	80
4	 **2d**	14.30	 **4d**	87
5	 **2e**	11.50	 **4e**	30
6	 **2f**	11.37	 **4f**	61
7	 **2g**	14.28	 **4g**	84
8	 **2h**	11.50	 **4h**	96
9	 **2i**	8.70	 **4i**	74
10	 **2j**	9.54	 **4j**	41

Table 1. *Continued ...*

Entry	Amines	*pKa* [a]	Products	Yields (%) [b]
11	 **2k**	10.36	 **4k**	51
12	 **2l**	11.38	 **4l**	68

a. *pKa* values of aliphatic and aromatic amines **2(a-l)** at 25 °C
b. Isolated yields.

(m, 2H, CH), 7.46-7.74 (m, 2H, CH). ^{13}C NMR [75 MHz]: δ = 52.12 (CH_2), 66.73 (CH_2), 71.39 (CH_2), 113.84 (CH), 123.65 (CH), 124.61 (CH), 125.85 (C), 127.16 (CH), 142.36 (C), 189.17 (C=S). HRMS (ESI$^-$): Anal. Calcd. for $[C_{12}H_{14}N_2OS_2]^-$: 266.0548; found: 266.0537.

3-(piperidin-1-ylmethyl)-1,3-benzothiazole-2-thione (**4b**): White solid. Mp = 159-161 °C. ^{1}H NMR [$CDCl_3$, 300MHz]: 5 = 1.18-1.22 (m, 2H, CH_2), 1.59 (q, 4H, CH_2), 2.63 (q, 4H, CH_2), 4.91 (s, 2H, CH_2), 7.32-7.43 (m, 2H, CH), 7.49-7.74 (m, 2H, CH). ^{13}C NMR [75 MHz]: δ = 24.61 (CH_2), 26.15 (CH_2), 53.34 (CH_2), 71.01 (CH_2), 113.80 (CH), 123.62 (CH), 124.59 (CH), 125.35 (C), 127.11 (CH), 142.38 (C), 189.11 (C=S). HRMS (ESI$^-$): Anal. Calcd. for $[C_{13}H_{16}N_2S_2]^-$: 264.0755; found: 264.0741.

3-(piperazin-1-ylmethyl)-1,3-benzothiazole-2-thione (**4c**): White solid. Mp = nd (not determined, decomposes above 221 ºC). ^{1}H NMR [$CDCl_3$, 300MHz]: 5 = 2.02-2.06 (m, 1H, NH), 2.69-2.74 (m, 8H, CH_2), 4.91 (s, 2H, CH_2), 7.32-7.43 (m, 2H, CH), 7.49-7.74 (m, 2H, CH). ^{13}C NMR [75 MHz]: δ = 45.29 (CH_2), 51.53 (CH_2), 70.69 (CH_2), 113.87 (CH), 123.69 (CH), 124.58 (CH), 125.81 (C), 127.18 (CH), 142.39 (C), 189.15 (C=S). HRMS (ESI$^-$): Anal. Calcd. for $[C_{12}H_{16}N_3S_2]^-$: 265.0707; found: 265.0711.

3-[(4-nitroanilino)methyl]-1,3-benzothiazole-2-thione (**4d**): Pale brown solid (87%). Mp = 197-199 °C. ^{1}H NMR [DMSO-*d6*, 300MHz]: 5 = 5.05 (s, 2H, CH_2), 7.06-7.11 (m, 1H, NH), 7.32-7.39 (m, 2H, CH), 7.51-7.53 (m, 2H, CH), 7.81-7.83 (m, 4H, CH). ^{13}C NMR [75 MHz]: δ = 60.75 (CH_2), 112.93 (CH), 116.71 (CH), 122.74 (CH), 123.98 (CH), 124.58 (CH), 125.25 (C), 127.18 (CH), 143.31 (C), 145.39 (C), 189.92 (C=S). HRMS (ESI$^-$): Anal.Calcd. for $[C_{14}H_{11}N_3O_2S_2]^-$: 317.0293; found: 317.0301.

3-{[2,6-dichloro-4-(trifluoromethyl)anilino]methyl}-1,3-benzothiazole-2-thione (**4e**): White solid (30%). Mp = 186-187 °C. ^{1}H NMR [DMSO-*d6*, 300MHz]: 5 = 5.05 (s, 2H, CH_2), 7.38-7.41 (m, 2H, CH), 7.48-7.56 (m, 2H, CH), 7.55-7.57 (m, 1H, NH), 7.83-7.87 (m, 2H, CH). ^{19}F NMR [300 MHz] δ = -63.22 (s, 2F). ^{13}C NMR [75 MHz]: δ = 60.73 (CH_2), 75.4 (C), 113.4 (C), (116.5, 120.1, 123.7, 127.3, q, ^1J = 273.58Hz -CF_3), 126.2 (CH), (133.8, 134.2, 134.7, 134.9, q, ^2J = 34.59Hz -CF_3), 136.7(C), 142.9 (CH), 151.4(C), 189.88 (C=S). HRMS (ESI$^-$): Anal.Calcd. for $[C_{15}H_9Cl_2F_3N_2S_2]^-$: 407.9536; found: 407.9521.

3-[(3,4,5-trifluoroanilino)methyl]-1,3-benzothiazole-2-thione (**4f**): White solid (61%). Mp = 187-188 °C. ^{1}H NMR [DMSO-*d6*, 300MHz]: 5 = 5.05 (s, 2H, CH_2), 6.38-6.41 (m, 2H, CH), 7.50-7.55 (m, 2H, CH), 7.53-7.57 (m, 1H, NH), 7.84-7.88 (m, 2H, CH). ^{19}F NMR [300 MHz] δ = -62.30 (s, 3F). ^{13}C NMR [75 MHz]: δ = 60.70 (CH_2), 77.2 (C), 113.6 (C), 121.6 - 125.2 (q, ^1J = 272.58Hz, CF_3; it was not possible to assign the other half of this quartet), 123.9 (CH), 127.2 (CH), 130.4 - 130.8 (q, ^2J = 32.0Hz), 140.0 (C), 141.9 (C), 150.0(C), 189.91 (C=S). HRMS (ESI$^-$): Anal .Calcd. for $[C_{15}H_9Cl_2F_3N_2S_2]^-$: 326.0159; found: 326.0142.

3-[(2,3,4,5,6-pentafluoroanilino)methyl]-1,3-benzothiazole-2-thione (**4g**): White solid (84%). Mp = 169.2 °C. ^{1}H NMR [DMSO-*d6*, 300MHz]: 5 = 5.05 (s, 2H, CH_2), 7.42-7.48 (m, 2H, CH), 7.52-7.55 (m, 1H, NH), 7.85-7.88 (m, 2H, CH). ^{19}F NMR [300 MHz] δ = -158.95 (t, 2F), -148.58 (t, 1F), -143.24 (d, 2F). ^{13}C NMR [75 MHz]: δ = 61.01 (CH_2), 76.4 (C), 113.1 (C), 143.4 (CH), 152.1 (C), 189.90 (C=S). It was not possible to assign the other carbons corresponding to this molecule. HRMS (ESI$^-$): Anal.Calcd. for $[C_{14}H_7F_5N_2S_2]^-$: 361.9971; found: 361.9959.

3-[(2-bromoanilino)methyl]-1,3-benzothiazole-2-thione (**4h**): White solid (96%). Mp = 156-158 °C. ^{1}H NMR [DMSO-*d6*, 300MHz]: 5 = 5.05 (s, 2H, CH_2), 6.73-6.77 (m, 1H, CH), 7.01-7.03 (m, 1H, CH), 7.17-7.21 (m, 1H, CH), 7.42-7.48 (m, 2H, CH), 7.52-7.54 (m, 1H, NH), 7.56-7.58 (m, 1H, CH), 7.83-7.87 (m, 2H, CH). ^{13}C NMR [75 MHz]: δ = 60.73 (CH_2), 112.91 (CH), 116.73 (CH), 122.76 (CH), 123.95 (CH), 124.56 (CH), 125.23 (C), 127.20 (CH), 143.30 (C), 145.41 (C), 190.01 (C=S). HRMS (ESI$^-$): Anal.Calcd. for $[C_{14}H_{11}BrN_2S_2]^-$: 351.9526; found: 351.9539.

3-[(4-methoxyanilino)methyl]-1,3-benzothiazole-2-thione (**4i**): White solid (74%). Mp = 182-183 °C. ^{1}H NMR [DMSO-*d6*, 300MHz]: 5 = 3.76 (s, 3H, O-CH_3), 5.05 (s, 2H, CH_2), 6.79-6.81 (m, 2H, CH), 7.13-7.15 (m, 1H, NH), 7.22-7.24 (m, 2H, CH), 7.36-7.38 (m, 2H, CH), 7.80-7.82 (m, 2H, CH). ^{13}C NMR [75 MHz]: δ = 55.33 (CH_3), 60.49 (CH_2), 112.69 (CH), 116.70 (CH), 122.71 (CH), 123.90 (CH), 124.55 (CH), 125.25 (C), 127.16 (CH), 143.33 (C), 145.37 (C), 189.04 (C=S). HRMS (ESI$^-$): Anal.Calcd. for $[C_{15}H_{14}N_2OS_2]^-$: 302.0548; found: 302.0531.

3-[(2-aminoanilino)methyl]-1,3-benzothiazole-2-thione (**4j**): White solid (41%). Mp = nd (not determined, decomposes above 210 ºC). ^{1}H NMR [DMSO-*d6*, 300MHz]: 5 = 4.02 (s, 2H, NH), 5.05 (s, 4H, CH_2), 6.73-6.77 (m, 3H, CH), 7.01-7.03 (m, 1H, CH), 7.38-7.41 (m, 4H, CH), 7.55-7.57 (m, 2H, NH), 7.83-7.87 (m, 4H, CH). ^{13}C NMR [75 MHz]: δ = 60.31 (CH_2), 112.55 (CH), 116.65 (CH), 122.76 (CH), 123.10 (CH), 124.50 (CH), 125.13 (C), 127.17 (CH), 143.31 (C), 145.39 (C), 189.14 (C=S). HRMS (ESI$^-$): Anal.Calcd. for $[C_{12}H_{18}N_4S_4]^-$: 466.0414; found: 466.0422.

3-[(1-naphthalene sulphonic acid-4-amino)methyl]-1,3-benzothiazole-2-thione (**4k**): Pink solid (51%). Mp = nd (not determined, decomposes above 210 ºC). ^{1}H NMR [DMSO-*d6*, 200MHz]: 5 = 5.05 (s, 2H, CH_2), 7.25-7.34 (m, 3H, CH), 7.39-7.45 (m, 2H, CH), 7.77-7.84 (m, 2H, CH), 7.90-7.92 (m, 1H, CH), 8.08-8.12 (m, 1H, CH), 8.20-8.22 (m, 1H, NH), 8.40-8.42 (m, 1H, CH), 9.26 (s, 1H, OH). ^{13}C NMR [75 MHz]: δ = 77.05 (CH_2), 107.71 (CH), 112.55 (CH), 116.65 (CH), 122.41 (CH), 122.76 (CH), 123.10 (CH), 124.50 (CH), 125.32 (C), 125.82 (CH), 126.13 (C), 127.17 (CH), 129.41 (C), 129.91 (C), 130.21 (CH), 143.31 (C), 145.39 (C), 189.14 (C=S). HRMS (ESI$^-$): Anal.Calcd. for $[C_{12}H_{18}N_4S_4]^-$: 402.0167; found: 402.0154.

3-[(3,3´-dimethylnaphthidine)methyl]-1,3-benzothiazole-2-thione (**4l**): Grey solid (68%). Mp = 187-188 °C. ^{1}H NMR [DMSO-*d6*, 200MHz]: 5 = 2.27 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 4.86-4.93 (m, 2H, NH_2), 5.05 (s, 2H, CH_2), 7.22-7.24 (s, 1H, CH), 7.26-7.28 (m, 1H, NH), 7.40-7.46 (m, 6H, CH), 7.80-7.86 (m, 3H, CH), 7.88-7.94 (m, 3H, CH), 8.06-8.08 (m, 1H, CH).
^{13}C NMR [75 MHz]: δ = 18.12 (CH_3), 18.31 (CH_3), 75.15 (CH_2), 118.71 (C), 116.65 (CH), 122.41 (CH), 122.76 (CH), 123.10 (CH), 124.50 (CH), 125.32 (C), 125.82 (CH), 126.61 (C), 128.55 (CH), 127.17 (CH), 129.41 (C), 129.91 (C), 131.21 (C), 143.31 (C), 139.39 (C), 191.33 (C=S). HRMS (ESI$^-$): Anal.Calcd. for $[C_{30}H_{25}N_3S_4]^-$: 491.1490; found: 491.1479.

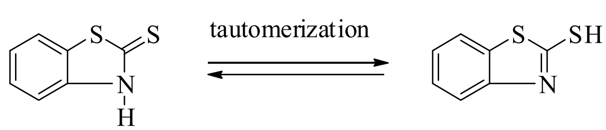
Antimicrobial evaluation

Agar well diffusion tests: With the purpose to determine the existence of antimicrobial activity of synthesized compounds **4**(**a-l**), agar well diffusion test was applied (Balouiri *et al*., 2016, Valgas *et al*., 2007, Magaldi, *et al*., 2004, Devillers *et al*., 1989). Stock solution of every compound had a concentration of 1500 μg/mL, and were prepared with sterile distillated water, dimethyl sulfoxide at 5–20% depending on their solubility and tween 20 at 1%. Seven-day-old cultures of *Cladosporium cladosporioides, Aspergillus niger* ATCC 16404, *Botrytis* sp. and *Rhizopus* sp. (PDA, 27 ± 1°C) were used to prepare inoculums at concentration between 0,4 x 10^4 - 5 x 10^4 CFU/mL to ensure reproducible values (NCCLS, 2002). Each conidial suspension was quantified with a Neubauer chamber. Inoculum's concentrations were verified by spread plate method, using PDA. These plates were incubated for 48 to 72 hours, at 27 ± 1°C, until colonies could be count. For the assay, 20 mL of PDA at 45 °C was mixed with 1 mL of inoculum and poured in a 90 mm Petri dish. 5 wells of 8 mm were cut in each plate and filled with 100 μL of the 2-MBT derivatives stock solutions (150 μg of pure compound). Wells of a control plate were filled with water and water + DMSO at 20% with Tween 20 at 1% (control A and B, respectively). All plates were done duplicated and were incubated for 48 hours at 27 ± 1°C and inhibitory halos formation were controlled. When an inhibitory halo was found, the compound was reported as positive (+), but when it was not present, it was reported as negative (-). The appearance of inhibition halos indicated that tested compounds would be active against the evaluated microorganism. Fluconazole (1500 µg/mL, water-soluble antifungal agent) was used as a reference drug.

RESULTS AND DISCUSSION

Synthesis

Twelve benzothiazole derivatives were prepared with affords moderate to good yields from formaldehyde, amines and 2-MBT. The synthesis of *3-[(amino)methyl]-1,3-benzothiazole-2-thione* **4(a-l)** by Mannich reaction was accomplished as presented in Fig. 1. In the first step it involves the nucleophilic addition of aliphatic or aromatic amines **2(a-l)** to formaldehyde (**1**) followed by dehydration to form the Schiff base. The Schiff base is an electrophile which reacts in the second step through an electrophilic addition on iminothiol tautomer of 2-MBT (**3**) containing an acidic proton. Finally, the compounds **4(a-l)** were cooled in water, filtered, and washed with ethanol. Synthesized compounds were obtained as pure crystals and were characterized by the basic analysis of the spectroscopic data obtained (HRMS, ^{1}H-NMR, ^{13}C-NMR, ^{19}F-NMR experiments). In particular, 2-MBT contain an extended ð-delocalized systems and present an iminothiol-thioamide tautomerism (Balestrero *et al.*, 1986; Hassan and Khan, 2021), shown below (Figure 2)
Following the procedure described by Holbová *et al.* (1976), the molecule of 2-MBT was substituted in the position 3 of iminothiol tautomer. These results confirm that the iminothiol tautomer is the predominant tautomer for Mannich reaction and *pKa* values of amines employed promote only the formation of monoderivative products, substituted in the position 3 of 2-MBT ring. All compounds were characterized by ^{1}H-RMN, ^{13}C-RMN and HRMS spectroscopic data, respectively. For aliphatic amines the ^{1}H-RMN spectrum ($CDCl_3$) showed a singlet at δ 4.91ppm confirmed signal for methylene protons N-$\underline{CH_2}$-NH-R, multiplets ranging from δ 7.80–7.30 ppm confirmed signal for aromatic protons, respectively. For aromatic amines the ^{1}H-RMN (DMSO-*d6*) spectrum showed a singlet at δ 5.05 ppm confirmed signal for methylene protons N-CH_2-NH-R, multiplets ranging from δ 8.12–6.86 confirmed aromatic protons and singlet ranges at δ 7.17-6.10 confirmed the presence of NH-R, respectively. Spectral data of ^{13}C and ^{19}F confirm the final structures for compounds **4(a-l)**.



iminothiol thioamide

Fig. 2. Prototropic equilibria iminothiol-thioamide of 2-MBT

Antimicrobial evaluation

The synthesized compounds were evaluated for their antifungal activity against *Cladosporium cladosporioides*, *Aspergillus niger* ATCC 16404, *Botrytis sp.* and *Rhizopus sp.* Agar well diffusion test was applied. A general view of the obtained results is exposed in Table 2. Compounds **4a-c**, **4e-g**, **4i** and **4l** showed antifungal activity against the four mentioned fungi, due to the presence of an inhibitory halo around the well with the corresponding compound. **4h** only presented an inhibitory zone in case of *C. cladosporioides*; so its activity is considered moderate to low. Compounds **4d**, **4j** and **4k** did not show activity against the molds used for the test. Fluconazole showed biological activity against *Aspergillus niger* ATCC 16404 and *Botrytis* sp.

Taking into account the results achieved, 8 of the 12 synthesized products have shown antifungal activity against filamentous fungi. Such promising substances should be studied as potential drugs to treat patients with pathogens caused diseases.

CONCLUSION

Twelve compounds **4(a-l)** were synthesized by Mannich reaction from formaldehyde, aliphatic and aromatic amines and 2-mercaptobenzothiazole. The yields obtained were satisfactory and the purity obtained for each product was >99 %. All compounds were characterized by nuclear magnetic resonance (^{1}H-RMN, ^{13}C-RMN, ^{19}F-RMN) and high-resolution mass spectrometry (HRMS) spectroscopic data. Synthesized products were evaluated against four different fungal species by agar diffusion test. The antifungal activity displaying different degree of antimicrobial activity, i.e. compounds **4a-c**, **4e-g**, **4i** and **4l** showed antifungal activity against

Table 2. Antimicrobial activity of synthesized compounds **4(a-l)** by agar well diffusion method

Compounds	*Cladosporium cladosporioides*	*Aspergillus niger*	*Botrytis* sp. ATCC 16404	*Rhizopus* sp.
4a	+	+	+	+
4b	+	+	+	+
4c	+	+	+	+
4d	-	-	-	-
4e	+	+	+	+
4f	+	+	+	+
4g	+	+	+	+
4h	+	-	-	-
4i	+	+	+	+
4j	-	-	-	-
4k	-	-	-	-
4l	+	+	+	+
Fluconazole	-	+	+	-
Control A	-	-	-	-
Control B	-	-	-	-

Positive (+): an inhibitory halo was found. Negative (-): inhibitory halo not present.
Compounds concentration: 1500 μg/mL. Fluconazole concentration: 1500 μg/mL.
Reading done at 48 h of incubation at 27°C ± 1°C
Culture media: potato dextrose agar (PDA)
Wells size: 8 mm

Cladosporium cladosporioides, *Aspergillus niger* ATCC 16404, *Botrytis* sp. and *Rhizopus* sp., while **4d**, **4j** and **4k** did not show activity against the molds used for the test. These synthesized products are promising and will continue to be studied as a part of our program aimed at the development of new heterocyclic compounds with potential antifungal activities.

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Conflict of Interest

There is no conflict of interest between the authors, all authors contributed directly to the article.

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