SYNTHESIS AND CHARACTERIZATION OF NOVEL SULFONAMIDES DERIVATIVES AND THEIR ANTIMICROBIAL, ANTIOXIDANT AND CYTOTOXICITY EVALUATION

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ABSTRACT. Five novel sulfonamides derivatives HR5-HR8 and HR14 were synthesized by sulfonylation of primary or secondary amine in the presence of base through nucleophilic substitution reaction. Structural elucidation was carried out through FT-IR, UV, 1H NMR, MS and elemental analysis. Prepared compounds were evaluated against pathogenic strains of bacteria (S. aureus and E. coli) and fungi (A. flavous and A. nyger). Results were compared against standard antifungal and bacterial drug already available in market (isoconazole and sulfmethoxazole). It was found that compound HR14 showed good activity with MIC 1.5 µg/mL and 2.0 µg/mL for S. aureus and E. coli, respectively. While HR5 showed best antifungal activity with zone of inhibition 27.2±0.12 mm (MIC: 5.25 µg/mL) and 18.1±0.12 mm (MIC: 12.5 µg/mL) against A. flavous and A. nyger, respectively. Synthesized compounds were also tested for their in vitro antioxidant activity by using DPPH. Amongst all compounds HR5 was found to have potential activity with 15.60% antioxidant activity at 6 mM concentration.

KEY WORDS: Sulfonamide, DPPH, AntiMICrobial activity

INTRODUCTION

Sulfonamides drugs have been used as preventive agents in chemotherapy against various diseases [1]. More than 30 drugs having sulfa drug as a functional group are in clinical use, such as antibacterial [2], antifungal [3], antiprotozoal [4], anti-inflammatory [5], and translational initiation inhibitors [6]. More recent use of sulfonamides are as an anticancer agent [7], antiviral HIV [8], and in Alzheimer’s disease [9]. They are used effectively for the treatment of ulcerative colitis [10], urinary, intestinal and ophthalmMIC infections and also for obesity [11]. Beside their vital role in human medicine they are also showing their promising importance in field of veterinary and agricultural sciences. Due to presence of SO2NH– group most important role of sulfonamide in medicinal field is as an antibacterial agent. Synthesis of bacterial DNA and RNA requires tetrahydrofolate as a co-factor, which is inhibited by sulfonamides, so production of new DNA and RNA dropped from lack of tetrahydrofolate which eventually decayed bacteria. Newer sulfonamides and their derivatives has obtained great attention in pharmaceutical field in order to compete life threatening issues caused by drug resistant strains of bacteria, i.e. Methicillin resistance as they have unusual ability of acclimatization against stress caused by antibiotics [12]. Disease causing organisms become much resistant when treated medically with routine antibiotic drug molecule, with appearance of additional species as per mutation, conjugation, transduction or transformation. So synthesis of new sulfonamides and
their derivatives have got more attention from researchers for its application in the field of medicine sciences and medical chemistry. In the present study five sulfonamide derivatives have been synthesized by the reaction of p-toluene sulfonyl chloride with NH₂ group containing drugs such as ceftriaxone, cefepime, nicotinamide (vitamin B), cefadroxil, and nimusulide, respectively and their biological activities were evaluated by using bacterial and fungal strains such as Escherichia coli, Aspergillus niger and Aspergillus flavus.

**EXPERIMENTAL**

1H NMR spectra were conducted on Bruker 400 MHz spectrometer in DMSO-d₆, with tetramethylsilane as internal standard. MS data was recorded on Finnigan MAT 112 mass spectrometer. Elemental analysis was performed by using Perkin Elmer elemental analyzer. Melting points were taken on Gallenhamp MP Apparatus MP70. Infrared spectra were recorded on Cary 630 Agilent FT-IR in the range between 4000-600 cm⁻¹. Absorption spectra were recorded by PGT90+ UV-Vis spectrophotometer.

**General procedure**

In this work efficient method based on Hinsberg test was used for preparation of sulphonamides, i.e. sulfonylation of primary or secondary amine in presence of base resulting in nucleophilic attack by amine. For sulfonylationtosyl chlorides were used [13] and base were used for neutralization of generated HCl, i.e. pyridine in synthesis of sulfonylmethylamide [14]. In the present work base sodium carbonate was used for neutralization of HCl. It was a one pot reaction, amine containing drug (0.001 M) in water was stirred and pH was noted, then equimolar sulfonyl chloride was added and mixture was allowed to stirrer for 2 hours and pH was monitored. Precipitates were separated by filtration and were purified by preparatory thin layer chromatography.

**Synthesis of (6R,7R)-7-(((Z)-2-(methoxyimino)-2-(2-(4-methylphenylsulfonyl)thiazol-4-yl)acetamido)-3-((2-methyl-5,6-dioxo-1,2,5,6-tetrahydro-1,2,4-triazin-3-yl)(thio)methyl)-8-methylene-3-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (HR5)**

![Chemical structure of HR5](image)

Title compound HR5 was prepared by above mentioned general procedure and was purified by using mobile phase DCM:EtOH, 50:50 with 83% yield. UV-Vis, λₘₚₚₚ: 270 nm. FT-IR (cm⁻¹): Bull. Chem. Soc. Ethiop. 2017, 31(3)
1178.19 (S=O str), 1037.18 (C=N str), 1633.80 (C=O), 846.97 (C=S str), 945.04 (S=N str), 3395.40 (–OH). 1H NMR (400 MHZ, DMSO-d₆, δ): 11.22 (1H, s, −COOH), 8.36-8.47 (1H, d, J = 6.5, −CH/NCO), 8.11 (1H, s, −CO/INCH), 7.39-7.47 (2H, d, m, ArH), 7.22 (1H, s, −CHS), 7.05-7.11 (2H, m, ArH), 5.68-5.76 (1H, d, J = 6.3, −CHHOCO), 5.47-5.50 (1H, d, J = 1.2, −CHH=CH−), 5.12-5.23 (1H, d, J = 6.2, −NCHS−), 4.70-4.73 (1H, d, J = 1.1, −CHH=CH−), 4.56 (2H, s,−CH−S=O str), 4.15 (1H, s, −NHSO−), 3.92 (3H, s, −OCH−), 3.47-3.56 (1H, d, J = 6.5,−CHH−S−), 3.31 (3H, s, −NCH2), 3.08-3.17 (1H, d, J = 6.3,−CHH−S−), 2.49 (3H, s, −ArCH3); MS (m/z, ESI): calcd. for C₈H₆N₂O₃S [M+H]+ 706.021012; found 706.021015. Anal. calcd. For C₈H₆N₂O₃S: C, 42.36; H, 3.4; N, 15.81; O, 20.32; S, 18.10. Found: C, 42.16; H, 3.46; N, 15.84; O, 20.17; S, 18.13.

**Synthesis of (6R,7R)-7-(Z)-2-(methoxymino)-2-(2-(4-methylphenylsulfonamido)thiazol-4-yl)acetamido)-3-(1-methylpyrrolidin-1-yl)-4-methyl)-3-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (HR6)**

Designated compound HR6 was prepared by following the general procedure mentioned earlier and was purified by using mobile phase DCM:EtOH, 40:60. Compound was obtained with a good yield, 85%. λmax 280 nm. FT-IR (cm⁻¹): 1158.15 (S=O str), 1045.15 (C=S str), 1649.55 (−N=C=O), 825.38 (C=S str), 930.60 (S=N str), 3378.15 (−OH). 1H NMR (400 MHZ, DMSO-d₆, δ): 8.01-8.03 (1H, d, J = 6.1, −CH/HOCO−), 7.62-7.65 (2H, d, J = 8.3, −ArH), 7.44 (1H, s, −C=CHS−), 7.32-7.35 (2H, d, J = 8.2, −ArH), 5.52-5.58 (1H, d, J = 6.3, −NCHHOCO−), 5.08-5.10 (1H, d, J = 5.9, −SC/INCO−), 4.28 (1H, s, −NHSO−), 4.01 (3H, s, −OCH−), 3.81 (2H, s, −CHCH2NCH), 3.61 (1H, s, −CH2CH2), 3.37-3.48 (4H, t, J = 7.5, CHNCH₂CH₂−), 3.20-3.29 (1H, d, J = 6.5,−CHH−S−), 3.07-3.14 (1H, d, J = 6.4,−CHH−S−), 2.49 (3H, s, −ArCH3), 1.44-1.60 (4H, Q, J = 6.9, CHCH₂CH₂CH₂−); MS (m/z, ESI): calcd. for C₁₈H₁₇N₂O₈S [M+H]+ 634.1094327; found 634.092741. Anal. calcd. for C₁₈H₁₇N₂O₈S: C, 49.20; H, 4.76; N, 13.24; O, 17.64; S, 15.5. Found: C, 49.35; H, 4.75; N, 13.23; O, 17.63; S, 15.16.

**Synthesis of N-tosylnicotinamide (HR7)**

Following above mentioned procedure labeled compound HR7 was purified by using mobile phase DCM:EtOH, 60:40 having 76% yield. λmax 285 nm. FT-IR (cm⁻¹): 1150.35 (S=O str), 1055.12 (C=N str), 1641.45 (C=O), 815.32 (C=S str), 930.16 (S=N str), 3388.12 (−OH). 1H NMR (400 MHZ, DMSO-d₆, δ): 9.24 (1H, s, −ArH), 8.84-8.88 (1H, d, J = 8.3, −ArH), 8.51-8.54 (1H, d, J = 8.2, −ArH), 8.15 (1H, s, −NHSO−), 7.95-7.97 (2H, d, J = 8.1, −ArH), 7.68-7.71 (1H, t, J = 6.4, −ArH), 7.40-7.42 (2H, d, J = 8.2, −ArH), 2.33 (3H, s, −ArCH3); MS (m/z, ESI): calcd. for C₁₅H₁₃N₂O₆S [M+H]+ 276.5352716; found 276.573781. Anal. calcd. For
The results are given in Table 1.

**Antioxidant activity**

*DPPH radical scavenging assay*: Using DDPH *in vitro* antioxidant activity of synthesized compounds was evaluated by a reported method [15]. All compounds were run in triplicate in order to produce precision of results. Trolox was used for standard curve. Using R² value relative concentrations of compounds were determined, and scavenging %, directly representing antioxidant activity, was determined using formula: Inhibition % = (1-sampleA/blank) × 100. The results are given in Table 1.
Table 1. Antioxidant activity of sulfonamide derivatives HR5-HR8 and HR14.

| Compound | % antioxidant activity |
|----------|------------------------|
|          | 4 mM | 6 mM |
| Trolox   | 1.53 | 12.87 |
| HR5      | 1.52 | 15.60 |
| HR6      | 1.47 | 15.19 |
| HR7      | 1.48 | 15.06 |
| HR8      | 1.26 | 14.17 |
| HR14     | 1.22 | 13.07 |

Biological activity

**Antibacterial activity.** Growth media used was Luria-Bertain broth as it is highly efficient in bacterial growth [16]. Media was prepared using 4.0 g of tryptone, 2.0 g of yeast extract and 4.0 g of sodium chloride in 400 mL distilled water. Value of pH of media was maintained at 7.0. Above mentioned media was autoclaved at 125 °C for 30 min. Sample solutions were prepared in 5-50 µg concentration range. Three test tubes were labeled for each bacterial strain, i.e. S. aureus and E. coli. 2 mL of LB Broth and 20 µL of bacterial strain were added in above sterilized tubes. After that stocks of 5, 10, and 20 µL containing 5, 12.5, and 50 µg were added in them. Then these tubes were incubated at 37 °C for 72 hours. After this OD of each medium and control medium were taken at 600 nm. Graph was plotted between concentration and OD of compounds showing a comparative study for synthesized compounds (Table 2).

Table 2. Determination of MIC µg/mL synthesized products against bacterial strains.

| S. No. | Name of compound | S. aureus µg/mL | E. coli µg/mL |
|--------|------------------|----------------|--------------|
| 1      | HR5              | 2              | 5            |
| 2      | HR6              | 6.5            | 8.5          |
| 3      | HR7              | 3              | 4.0          |
| 4      | HR8              | 2.5            | 3.0          |
| 5      | HR14             | 1.5            | 2.0          |
| 6      | Sulfmethoxazole  | 0.2            | 0.04         |

**Antifungal activity.** Antifungal activity of compounds was evaluated by performing well diffusion test [17-21] using PDA (potato dextrose agar). A 24 h yeast culture of PDA was used to prepare inoculum. Sterile saline solution (0.85%) was used for making suspension. Spectrophotometer was used to adjust turbidity of above suspension at 600 nm for getting final concentration matching with 0.5 McFarland standard. Agar medium was autoclaved for 30 min at 120 °C then cooled at 50 °C and inoculated with 1ml of above suspension having absorbance 0.5 McFarland. This inoculated medium were then poured into all assay plates 9cm in diameter and were allowed to cool down until solidified. Upon solidification, equidistance four wells 6mm in diameter were cut out of agar 6 µL of medium was plotted into these wells having synthesized compounds. These plates were than incubated at 27 °C for 48 h. MIC values in µg/mL and zone of inhibition in mm were calculated for each compound, comparing it with standard antifungal isonazol (ISC) in concentration 1.0 µg/mL in each plate as +ve control. Results are given in Table 3 and 4.

**Cytotoxicity test.** In vitro, cytotoxicity test was performed using Vero cell line. Assay was based on protocol described by Borenfreund and Puerner (1984). 10% FBS (Fetal Bovine Serum) containing Trypsin enzymes were used for cell growth in 96-well plates for 24 hours. After that 100 µL of each sample and standard was loaded in above plate. MIC values were determined by comparison to doxorubicin hydrochloride as a reference drug. Two fold dilutions of test
compounds and doxorubicin were prepared in ethanol (1 mL). Each dilute was finally added to media at room temperature giving a final concentration of 100, 50, 12.5 µg mL⁻¹. This loaded plate was incubated for 48 hours at 37 °C, then natural red dye 10 µL (40%) was introduced in all wells and incubated at same temperature for 4 hours. Then plate was washed two times with PBS and finally one time with acidified ethanol. Absorbance was recorded at 540 nm in a Microtiter plate reader spectrophotometer. Activity of each well was found using given formula and is presented in Table 5. Cytotoxicity of sample = (1-Experimental well abs/ abs of negative control) x100.

Table 3. Determination of MIC µg/mL products against fungal strains.

| S. No. | Name of compound | A. flavous | A. niger |
|--------|------------------|------------|---------|
| 1      | HR5              | 5.25       | 12.5    |
| 2      | HR6              | 8.50       | 14.5    |
| 3      | HR7              | 12.5       | 30.0    |
| 4      | HR8              | 50.0       | 65.5    |
| 5      | HR14             | 7.50       | 13.0    |
| 6      | Isoconazole      | 0.50       | 0.76    |

Table 4. Diameter of zone of inhibition (mm±SD).

| S. No. | Name of compound | A. flavous | A. niger |
|--------|------------------|------------|---------|
| 1      | HR5              | 27.2±0.12  | 18.1±0.12 |
| 2      | HR6              | 26.5±0.30  | 16.3±0.33 |
| 3      | HR7              | 18.9±0.11  | 14.9±0.22 |
| 4      | HR8              | 12.4±0.22  | 10.6±0.10 |
| 5      | HR14             | 25.5±0.10  | 17.1±0.11 |
| 6      | Isoconazole      | 30         | 29.5    |

Table 5. Cytotoxicity values of sulfonamide derivatives.

| Compound            | Absorbance | % Activity |
|---------------------|------------|------------|
|                     | 100        | 50         | 12.5       | 100 | 50 | 12.5 |
| HR5                 | 0.89       | 0.90       | 0.987      | 44  | 40 | 5.2  |
| HR6                 | 0.90       | 0.94       | 0.96       | 40  | 24 | 16   |
| HR7                 | 0.94       | 0.98       | 0.99       | 24  | 8.0| 4.0  |
| HR8                 | 0.94       | 0.97       | 0.99       | 24  | 12 | 4.0  |
| HR14                | 0.90       | 0.94       | 0.99       | 40  | 24 | 4.0  |
| Doxorubicin hydrochloride | 0.76 | 0.89 | 0.93 | 96  | 44 | 28   |

RESULT AND DISCUSSION

A series of five sulfonamides were synthesized in aqueous basic media by simple reaction of five amino group containing drugs; ceftriaxone, cefepime, nicotinamide (vitamin B), cefadroxil and nimsulide with paratoluenesulphonyl chloride with continuous stirring and details of reaction conditions are explained in experimental section and synthetic pathway of sulfonamides is explained in general procedure. The compounds were obtained in good to excellent yield (55–87%). Elemental analysis was performed for the conformation of all the compounds and measurement of absorption maximum (λmax) provided the justification. The synthesized compounds were characterized by FT-IR; the characteristics band at 1148–1155.5 cm⁻¹ of S=O stretching and 1048-1055 cm⁻¹ for (C-N) and 813-814 cm⁻¹ (C-S) and 930-958.9 cm⁻¹ (S-N) for all compounds reveals the formation of sulfonamides. Mass spectral data of all synthesized compounds was obtained by ESI-MS. The molar mass for compound HR5, C25H24N8O9S4, was originated as 708.77(calcd. 708.05). Correspondingly, observed mass for compound HR6,
C_{3b}H_{12}N_{2}O_{2}S_{2} was found as 634.13 (calcd. 634.75) and it proved the formation of the desired product. The prominent peaks for compounds, HR7 and HR8, were recorded at m/z 122.05 and 363.09 for stable fragments [C_{6}H_{12}N_{2}O]^{+}, [C_{10}H_{13}N_{2}O_{3}S]^{+}, respectively. Observed and calculated molar masses of compounds HR7 and HR8 were found as 276.07 (calcd. 276.31), and 517.10 (calcd. 517.57), respectively. The major peak for compound HR14 was noticed at m/z 462.06 and showed good agreement with calculated molecular masses of concerned compound. The structures of all the compounds were also confirmed by ¹H NMR by dissolving in DMSO. ¹H NMR spectra of compounds HR5-HR8, all ArCH₃ showed their cheMICal shift values from 21.39 to 21.84 ppm, and found to be very much similar to the literature values. In HR5 and HR6 two methyl signals, i.e. -NCH₃ and -OCH₃ were also recorded at 38.65, 60.21 ppm and 50.39, 63.99, respectively. The cheMICal shift value of -OCH₃ was observed on downfield side than -NCH₃ due to strong electron withdrawing influence of oxygen than nitrogen. In HR7 a prominent peak of methyl (C1) was noticed at 21.39 ppm. In this molecule there are two rings, one of them is attached with methyl and second ring contains nitrogen. In second ring cheMICal shift values of C10, C12 were found on downfield side due to electron withdrawing influence of nitrogen and peaks of C2, C4 were appeared on high field side due to the electron donating effect of -CH₃ group. In HR8 a small peak was noticed at 174.17 ppm due to carbon atom of – COOH group.

Synthesized compounds were screened for their antibacterial and antifungal activities using sulfmethoxazole and isoconzol as reference antibacterial and antifungal agents. All developed compounds showed moderate to good activity for both bacterial and fungal strains but compound HR14 exhibited excellent activity against the E. coli and S. aureus (MIC 1.5 and 2.0) and compound HR5 showed good activity against A. Flavus and A. Nyger (MIC 5.25 and 12.5). Synthesized compounds were also screened for their antioxidant activity. Compound HR5 showed excellent and pronounced activity at 4 mM concentration. The MIC values and zone of inhibitions are presented in Table 1-4. Cytotoxicity evaluation clearly shows that developed sulfonamides exhibited poor activity than standard drug, i.e. doxorubicin. Cytotoxicity values are presented in Table 5.

CONCLUSION

Five novel sulfonamides derivatives HR5-HR8 and HR14 were synthesized and evaluated for their antiMicrobial, antioxidant and cytotoxicity test. Most of the synthesized compounds showed promising antiMicrobial and antioxidant activity, suggesting a possible clinical significance of novel compounds. Compound HR14 showed remarkable antiMicrobial results, but compound HR5 was found to have potential antioxidant activity. However their cytotoxic effects are not so pronounced.

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REFERENCES

1. Hanch, C.; Sammes, P.G.; Taylor, J.B. Comprehensive Medicinal Chemistry, Vol. 2, Pergamon Press: Oxford; 1990, chap. 7.1.
2. Kanda, Y.; Kawanishi, Y.; Oda, K.; Sakata, T.; Mihara, S.; Asakura, K.; Kanemasa, T.; Ninomiya, M.; Fujimoto, M.; Kanoike, T. Synthesis and structure-activity relationships of potent and orally active sulfonamide ETB selective antagonists. Bioorg. Med. Chem. 2001, 9, 897-907.
3. Stokes, S.S.; Albert, R.; Buurman, Ed.T.; Andrews, B.; Shapiro, A.B.; Green, O.M.; McKenzie, A.R.; Otterbein, L.R. Inhibitors of the acetyltransferase domain of N-
acetylglucosamine-1-phosphate-uridylyltransferase/glucosamine-1-phosphate acetyltransferase (GlmU). Part 2: Optimization of physical properties leading to antibacterial arylsulfonamides. *Bioorg. Med. Chem. Lett.* 2012, 22, 7019-7023.

4. Chibale, K.; Haupt, H.; Kendrick, H.; Yardley, V.; Saravanamuthu, A.; Fairlamb, A.H.; Croft, S.L. Antiprototozoal and cytotoxicity evaluation of sulfonamide and urea analogues of quinacrine. *Bioorg. Med. Chem. Lett.* 2001, 11, 2655-2657.

5. Rahavi Ezabadi, I.; Camoutenis, C.; Zoumpoulakis, P.; Geronikaki, A.; Soković, M.; Glamočilija, J.; Ćirić, A. Sulfonamide-1,2,4-triazole derivatives as antifungal and antibacterial agents: Synthesis, biological evaluation, lipophilicity, and conformational studies. *Bioorg. Med. Chem.* 2008, 16, 1150-1161.

6. Kennedy, J.F.; Thorley, M. *Pharmaceutical Substances*, 3rd ed., Kleeman, A.; Engel, J.; Kutscher, B.; Reichert, D. (Eds.), Thieme: Stuttgart, 1999.

7. Lasensibilidad a la fluconazol de Candida albicans frente a los de uso comercial. *Diabetes*. 1999.

8. Magaldi S.; Zoumpoulakis, P.; Geronikaki, A.; Soković, M.; Glamočilija, J.; Ćirič, A. Sulfonamide-1,2,4-triazole derivatives as antifungal and antibacterial agents: Synthesis, biological evaluation, lipophilicity, and conformational studies. *Bioorg. Med. Chem.* 2008, 16, 1150-1161.

9. Kennedy, J.F.; Thorley, M. *Pharmaceutical Substances*, 3rd ed., Kleeman, A.; Engel, J.; Kutscher, B.; Reichert, D. (Eds.), Thieme: Stuttgart, 1999.

10. Lasensibilidad a la fluconazol de Candida albicans frente a los de uso comercial. *Diabetes*. 1999.

11. Levin, J.I.; Chen, J.M.; Du, M.T.; Nelson, F.C.; Killar, L.M.; Skala, S.; Suny, A.; Jin, G.; Cowling, R.; Barone, D.; March, C.J.; Mohler, K.M.; Black, R.A.; Skotnicki, J.S. Anthranilate sulfonamide hydroxamate TACE inhibitors. Part 2: SAR of the acetylenic P1’ group. *Bioorg. Med. Chem. Lett.* 2002, 12, 1199-1202.

12. Livermore, D.M. Antibiotics resistance in staphylococci. *Int. J. Antimicrobiol. Agents* 2000, 16, 3-10.

13. Whitaker, D.T.; Whitaker, K.S.; Johnson, C.R.; Haas, J. *p-Toluenesulfonyl Chloride. Encyclopedia of Reagents for Organic Synthesis*. John Wiley and Sons: New York; 2006; DOI: 10.1002/047084289X.r136.pub2.

14. Online Publication, Working with hazardous chemicals. *Organic Syntheses*, Coll. Vol. 4, p. 943 (1963); Vol. 34, p. 96 (1954). DOI:10.15227/orgsyn.034.0096.

15. Mohammad, H. Natural and synthetic flavonoid derivatives with potential antioxidant and anticancer activities. *PhD Dissertation*, Chemie, Pharmazie, Bio- und Werkstoffwissenschaften der Universität des Saarlandes, Saarbrücken, Germany, 2009.

16. Sezonov, G.; Joseleau-Petit, D.; D'Ari, R. *Escherichia coli* physiology in Luria-Bertani broth. *J. Bacteriology* 2007, 189, 8746-8749.

17. Magalía, S.; Camero, T. Susceptibilidad de Candida albicans ‘invitro’ mediante los pozos de difusión. *Boletín Venezolano de Infectología* 2000, 7, 5-8.

18. Magalí, S.; Camero, T.; Mata, S.; Ortega-Medrano, E.; Arroyo-Espinosa, D.I. Pruebas de sensibilidad de Candida albicans frente a los de uso comercial. *Boletín de Sociedad Venezolana de Microbiología* 1998, 18, 16-20.

19. Magalí, S.; Mata, S.; Camero, T.; Marchan, C.; Hartung, C. Determinación de la sensibilidad antifúngica de agentes de cromosómicos mediante la técnica de los pozos de difusión. *Antibióticos e Infección* 1999, 7, 17-20.

20. Magalí, S.; Mata, S.; Hartung, C.; Verde, G.; Deibis, L.; Rollán, Marchan, Y. ‘In vitro’ susceptibility of 137 Candida sp. isolates from HIV positive patients to several antifungal drugs. *Mycopathologia* 2000, 149, 63-68.

21. Magalí, S.; Rios, A.; Hartung, C.; Verde, G.; Spencer, L.; Mata, S. In vitro susceptibility of fluconazole of Candida spp. isolates comparing three different methods. *J. Mycol. Med.* 2001, 11, 123-126.