

Synthesis, Characterization and Antimicrobial Activity of New Pyrrole Derivatives

Akbar Idhayadhulla, Radhakrishnan Surendra Kumar, and Abdul Jamal Abdul Nasser*

P.G. & Research Dept. of chemistry, Jamal Mohamed College, Tiruchirappalli- 620020, Tamil Nadu, India.
jamal_abdulchem@gmail.com

Received April 12, 2011; accepted August 9, 2011

Abstract. Here we describe pyrrole derivatives with potent antibacterial and antifungal activity. A new series of pyrrole **3a-e** derivatives were synthesized using standard amination reactions. All the compounds presented here were obtained with high yields and under easy experimental conditions. Synthesized compounds were characterized by IR, ^1H NMR, ^{13}C NMR, mass spectra and mass spectral fragmentation. Synthesized compounds were screened against *E. coli* and *S. aureus* for antibacterial activity, as well as against *A. niger* and *C. albicans* for antifungal activity. We were able to obtain compounds with higher or equal potency to the reference compounds (Ciprofloxacin and Clotrimazole). Our data shows that a 4-hydroxyphenyl ring in our most potent compound seems to be responsible for antifungal activity against *C. albicans*. Incorporation of a 4-hydroxyphenyl ring as a pharmacophoric feature against *C. albicans* is a promising prospect. **Key words:** Pyrrole derivative, antimicrobial activity, structure-activity relationship.

Resumen. Se describe la síntesis de una nueva serie de derivados pirrólicos con potente actividad antibacteriana y antifúngica. La serie de compuestos **3a-e** se sintetizó empleando reacciones de afinación estándar. Todos los compuestos preparados se obtuvieron en elevados rendimientos y bajo fáciles condiciones experimentales. Estos compuestos se caracterizaron por IR, RMN ^1H , RMN ^{13}C , y su estudio de fragmentación en espectrometría de masas. Los compuestos sintetizados se evaluaron en su actividad antibacteriana contra *E. coli* y *S. aureus*, y en su actividad antifúngica contra *A. niger* y *C. albicans*. Se lograron obtener compuestos con una potencia mayor o igual a los compuestos de referencia: ciprofloxacina y clotrimazol. Los datos muestran que el anillo 4-hidroxifenilo en el compuesto más activo parece ser el grupo farmacóforo responsable de la actividad antifúngica contra *C. albicans*.

Palabras clave: Derivados pirrólicos, actividad antimicrobiana, relación estructura-actividad.

Introduction

Pyrrole heterocyclic derivatives were reported as having important synthetic and biological activities [1, 2] such as COX-1/COX-2 inhibitors [3] and cytotoxic activity against a variety of marine and human tumour models [4]. Thiazole derivatives also display a wide range of biological activities such as anesthetic [5] and anti-inflammatory [6] properties. Based on this observation, we are interested in preparing the biological behaviours of these title compounds. The above title compounds have not been published before, but previously reported methods appeared to meet our requirements. Therefore we synthesized novel pyrrole derivatives and screened their antimicrobial activity.

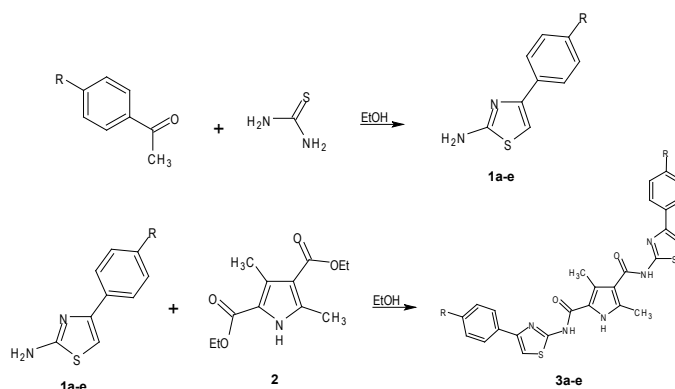
Results and Discussion

Chemistry

The general procedure for the synthesis of compounds **3a-e** and the compound 4-phenyl-1,3-thiazol-2-amine (**1a**) was prepared according to the method shown in the literature [7, 8].

Diethyl 3,5-dimethyl-1*H*-pyrrole-2,4-dicarboxylate **2** is prepared from the Fischer and Noller condensation method [9]. The general procedure for the synthesis of compounds **3a-e**, 3,5-dimethyl-*N*²,*N*⁴-bis(4-phenylthiazol-2-yl)-1*H*-pyrrole-2,4-dicarboxamide (**3a**) is prepared from amination method [10, 11]. The reaction sequence is outlined in **Scheme 1**. Physicochemical data of compounds **3a-e** are summarized in Table

1. The IR spectrum of **3a** shows absorption bands at 3350, 1680.16 and 619.11 cm^{-1} , corresponding to NH in pyrrole ring, CONH and the C-S-C group, respectively. The ^1H NMR spectrum (Figure 3) of compound **3a** shows the signals at δ 11.59 and 12.93, corresponding to the NH proton in the pyrrole ring and CONH protons, respectively. The ^{13}C NMR spectrum (Figure 4) of compound **3a** shows the carbon signals at δ 168.50 and 103.86, corresponding to CONH and CH carbons in the thiazole ring. The mass spectrum (EI-MS) of compound **3a** shows the molecular ion peak at m/z 499.82(M^+ , 87%), which is confirmed as the molecular weight of compound **3a**. Figure 5 and Figure 6 indicate the mass spectrum and mass spectral fragmentation of compound **3a**.



Scheme 1. Synthetic route of compounds **3a-e**.

Table 1. Physicochemical data of the compounds **3a-e**.

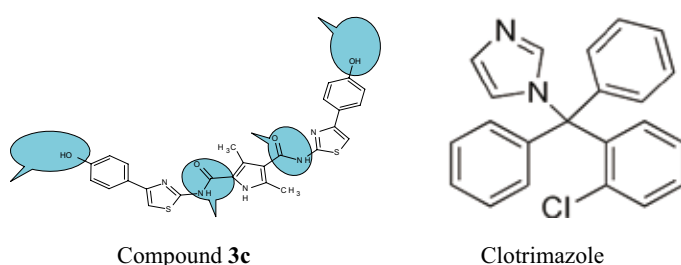
Comp. No.	R	mp °C	mw	Yield %	Color	Solvent	Reaction Time	Reaction Temperature (°C)
3a	—H	113	499.11	87	Yellow solid	EtOH	5h	Reflux,65
3b	—Cl	124	568.64	89	Pale yellow solid	EtOH	6h	Reflux,65
3c	—OH	120	531.10	81	Pale yellow solid	EtOH	5h	Reflux,65
3d	—NO ₂	123	589.08	86	Light brown solid	EtOH	7h	Reflux,65
3e	—OCH ₃	118	559.65	88	Yellow solid	EtOH	5h	Reflux,65

Biological activities

Antibacterial activity. Compounds **2** and **3a-e** were screened for antibacterial activity. The synthesized compound **3d** has equipotent activity compared with Ciprofloxacin against *E. coli* and *S. aureus* at a concentration of 100 µg/mL. Compound **2** has low activity compared with compounds **3a-e** and the reference compound at a concentration of 100 µg/mL. The bacterial zones of inhibition (mm) values are summarized in Table 2. Figure 1 indicated the antibacterial activity variation of compounds **2** and **3a-e**.

Antifungal activity. Compounds **2**, **3a-e** were screened for antifungal activity. The compound **3e** has equipotent activity compared with the reference drug (Clotrimazole) against *A. niger* and *C. albicans*. The compound **3c** and **3d** are highly active compared with the reference compound against *C. albicans* at a concentration of 100µg/mL. The fungal zones of inhibition (mm) values are summarized in Table 3. Figure 2 indicates the antifungal activity variation of compounds **2** and **3a-e**.

Structure-activity relationship



A key structural feature that differentiates our active compound, **3c**, from its inactive analogues is the presence of a 4-hydroxyphenyl ring. This functional group seems to be essential for antifungal activity against *C. albicans* at a concentration

of 100 mg/mL. Compound **3c** is highly active compared with standard Clotrimazole. Incorporation of a 4-hydroxyphenyl ring into the next generation of compounds, with the aim of antifungal activity against *C. albicans*, appears attractive to define its pharmacophoric importance.

Table 2. Antibacterial data of the synthesized compounds **2**, **3a-e**^a.

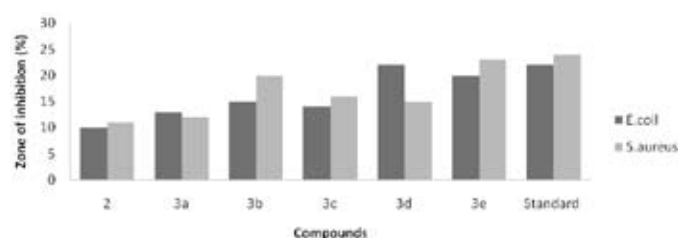
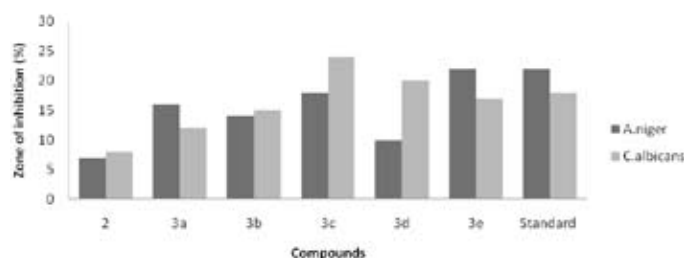
Compounds	<i>E.coli</i>	<i>S.aureus</i>
2	10	11
3a	13	12
3b	15	20
3c	14	16
3d	22	15
3e	20	23
Standard	22	24

^a Zone of inhibition was measured at (mm) at concentration of 100µg/mL, Ciprofloxacin is used as the standard.

Table 3. Antifungal data of the synthesized compounds **2**, **3a-e**^a.

Compounds	<i>A.niger</i>	<i>C.albicans</i>
2	7	8
3a	16	12
3b	14	15
3c	18	24
3d	10	20
3e	22	17
Standard	22	18

^a Zone of inhibition was measured at (mm) at concentration of 100µg/mL. Clotrimazole was used as a standard.

**Figure 1.** Antibacterial activity of compounds **2** and **3a-e**.**Figure 2.** Antifungal activity of the compounds **2**.

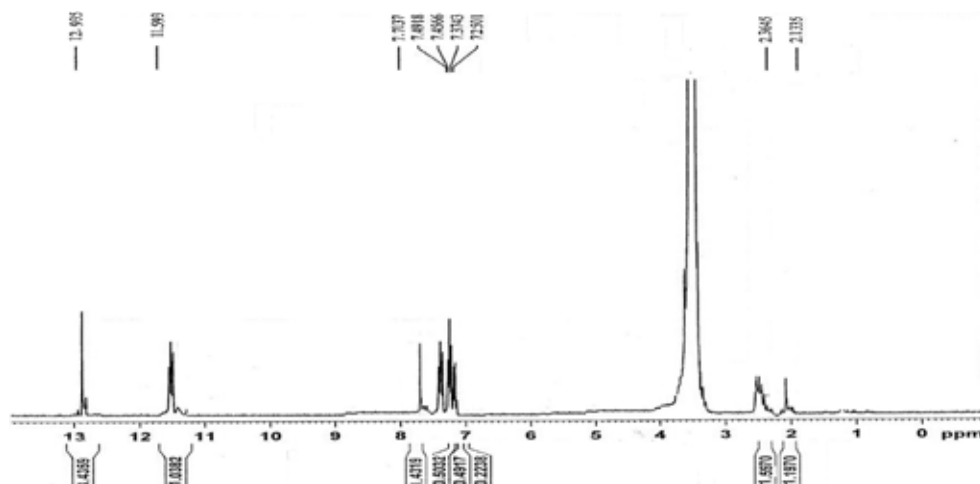


Figure 3. ^1H NMR spectra of compound **3a**.

Conclusion

This paper describes the synthesis of a new series of pyrrole-connected with thiazole derivatives through a cyclization method and in one-pot reaction procedure. The methodology was previously reported, but the target molecules are newly synthesized compounds at a concentration of 100 $\mu\text{g}/\text{mL}$. Compound **3d** had equipotent activity compared with the standard against *E. coli* at a concentration of 100 $\mu\text{g}/\text{mL}$, and compound **3c** was highly active against standard Clotrimazole against *C. albicans* at a concentration of 100 $\mu\text{g}/\text{mL}$.

Experimental

Melting points were recorded in open capillary tubes and were uncorrected. The IR spectra (KBr) were recorded in KBr on

a shimadzu 8201pc (4000-400 cm^{-1}). The ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker DRX-300 MHz. The Elemental analysis (C, H, and N,) were recorded using an Elementer analyzer model (Varian EL III). The purity of the compounds was checked by thin layer chromatography (TLC) with silica gel plates

4-Phenyl-1,3-thiazol-2-amine (1a)

To prepared the mixture of resublimed iodine (2.54 g, 0.01 mol), acetophenone (0.01 mol) and thiourea (3.19 g, 0.02 mol), followed by heating the mixture overnight in an oil bath at 100 $^\circ\text{C}$. After cooling, the mixture was triturated with diethyl ether (50 mL) to remove any unreactant iodine and acetophenone. The solid residue was poured in cold distilled water (200 mL) and treated with 25 % aqueous ammonium hydroxide (pH 9-10). The precipitate was collected and purified from

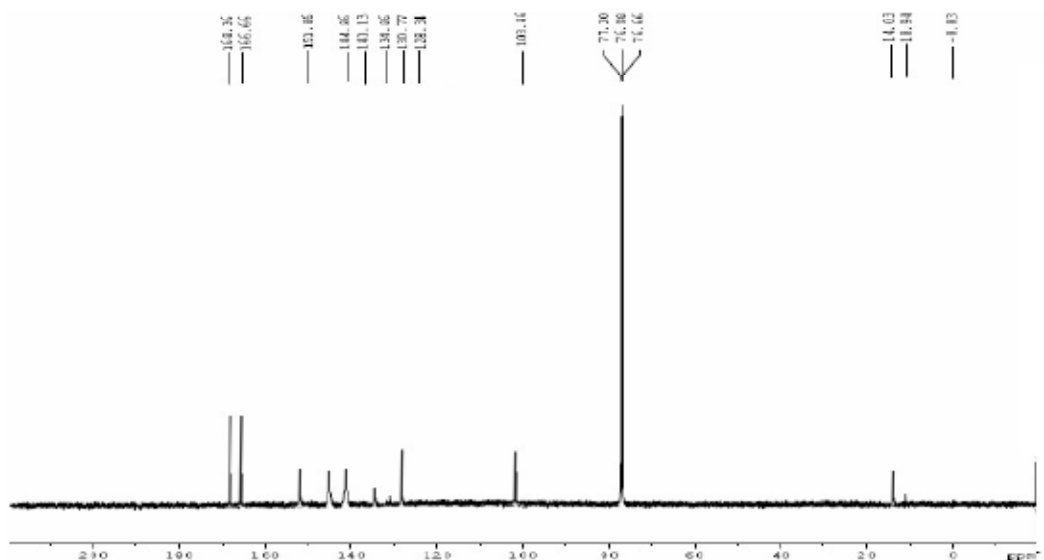


Figure 4. ^{13}C NMR spectra of compound **3a**.

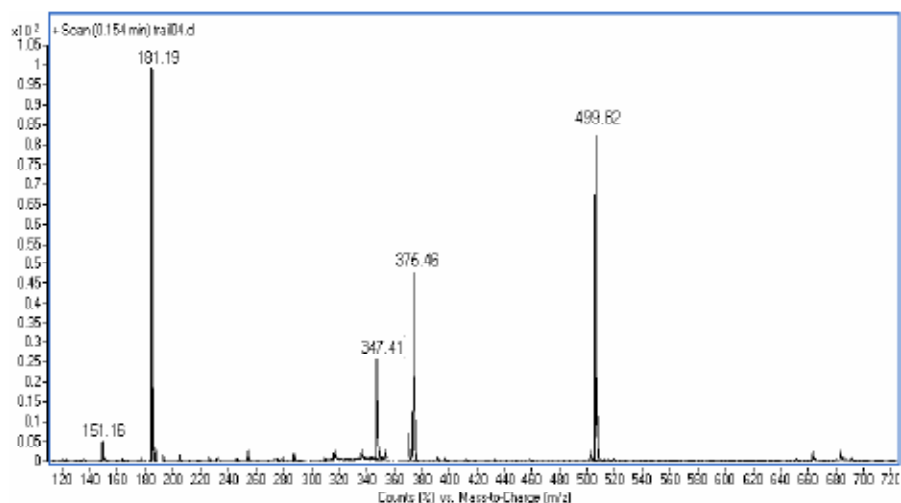


Figure 5. Mass spectra of compound 3a.

hot ethanol. Above procedure was followed by all remaining compounds **1b-1e**.

3,5-Dimethyl-*N*²,*N*⁴-bis(4-phenylthiazol-2-yl)-1*H*-pyrrole-2,4-dicarboxamide (**3a**)

A mixture of 2,4-dimethyl-3,5-dicarboxypyrrole **2** (2.39g, 0.01 mol), 2-amino-4-phenyl-thiazole **1a** (3.5 g, 0.02 mol) in ethanol (30mL), the reaction mixture was heated and refluxed for 5h, then cooled and poured into water. The resulting solid was filtered off, washed with water, dried and recrystallized from ethanol. Above procedure was followed by all remaining compounds **3b-e**.

IR (cm⁻¹): 3350.2 (NH), 3028.38 (Ph-CHstr), 2752.23 (2-CH₃), 1680.16 (OCNH), 1510.56 (C=N in Thiazole ring), 619.11 (C-S-C); ¹H NMR (DMSO-*d*₆): 12.93(s, 1H, OCNH),

11.59 (s, 1H, NH), 7.79 (s, 1H, CH in thiazole ring), 7.26-7.40 (m, 5H, Ph-H), 2.37(s, 3H, C3-CH₃), 2.14(s, 3H, C5-CH₃); ¹³C NMR (DMSO-*d*₆): 168.36(CONH), 166.66 (NH-C), 151.86 (Ph-C in Thiazole), 144.68 (C3,C5-CH₃), 141.13-128.34 (Ph), 103.86 (CH-Thiazole), 14.03 (C5-CH₃), 10.94 (C3-CH₃). EI-MS, *m/z* (M⁺, Relative intensity %) : 499.82 (M⁺, 83%), 375.46, 347.41, 181.19 (100%), 151.16. Elemental Analysis: C₂₆H₂₁N₅O₂S₂, Calc. C 62.50, H 4.24, N 14.02, S 12.84, Found C 62.51, H 4.21, N 12.88, S 12.82.

*N*²,*N*⁴-bis(4-(4-chlorophenyl)thiazol-2-yl)-3,5-dimethyl-1*H*-pyrrole-2,4-dicarboxamide (**3b**)

IR (cm⁻¹): 3350.21 (NH), 3028.38 (Ph-CHstr), 2752.23 (CH₃), 1680.16 (OCNH), 1510.42 (C=N in Thiazole ring), 837.32 (C-Cl), 619.11(C-S-C); ¹H NMR (DMSO-*d*₆): 12.84 (s, 1H,

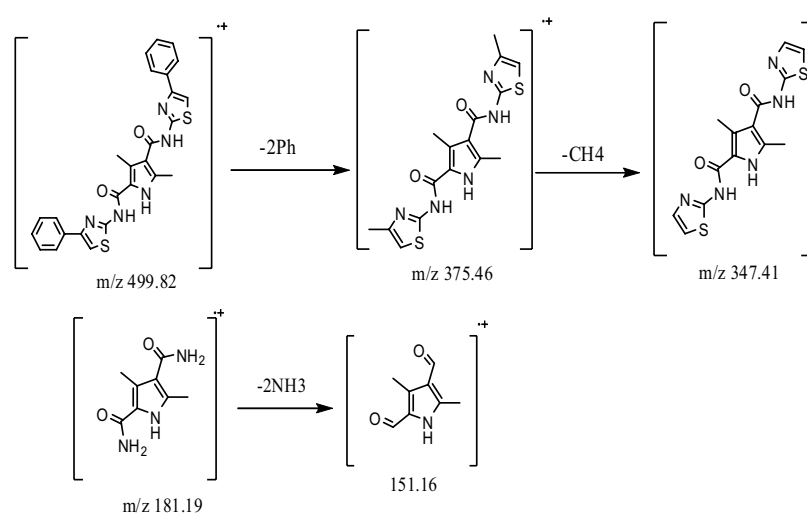


Figure 6. Mass spectral fragmentation of compound 3a.

OCNH), 11.66 (s,1H, NH), 7.66 (s,1H, CH in thiazole ring), 7.53-7.89 (dd, 4H, Ph-H), 2.29 (s, 3H, C5-CH₃), 2.10 (s, 3H, C3-CH₃); ¹³C NMR (CDCl₃): 162.36 (CONH), 164.66 (NH-C), 152.86 (Ph-C in Thiazole), 143.68 (C3, C5-CH₃), 134.86 (C-Cl), 130.77-128.34 (Ph), 105.86 (CH-Thiazole), 14.03 (C5-CH₃), 10.94 (C3-CH₃). EI-MS, *m/z* (M⁺, Relative intensity %): 568.20 (M⁺, 13%), 247.41, 264.30, 181.19 (100%), 167.16, 153.13, 67.08. Elemental Analysis: C₂₆H₁₉Cl₂N₅O₂S, Calc. C 54.93, H 3.37, N 12.32, S 11.28, Found C 54.90, H 3.36, N 12.30, S 11.27.

*N*²,*N*⁴-bis(4-(4-hydroxyphenyl)thiazol-2-yl)-3,5-dimethyl-1*H*-pyrrole-2,4-dicarbox amide (**3c**)

IR (cm⁻¹): 3350.2 (NH), 3028.38 (Ph-CHstr), 2752.23 (CH₃), 1680.16 (OCNH), 1465.21 (OH), 1510.28 (C=N in Thiazole ring), 619.11 (C-S-C); ¹H NMR (DMSO-*d*₆): 12.90 (s,1H, OCNH), 11.57 (s,1H, NH), 9.40 (C-OH), 7.75 (s,1H, CH in thiazole ring), 7.26-7.40 (dd, 4H, Ph-H), 2.36 (s, 3H, C3-CH₃), 2.16 (s, 3H, C5-CH₃); ¹³C NMR (CDCl₃): 168.30 (CONH), 166.61 (NH-C), 151.86 (Ph-C in Thiazole), 158.27 (C-OH), 143.98 (C3, C5-CH₃), 141.12-128.24 (Ph), 103.80 (CH-Thiazole), 14.13 (C5-CH₃), 10.91(C3-CH₃). EI-MS, *m/z* (M⁺, Relative intensity %): 531.22 (M⁺, 21%), 419.60, 347.41, 319.16 (100%), 123.10, 67.08. Elemental Analysis: C₂₆H₂₁N₇O₆S₂, Calc. C 58.74, H 3.98, N 13.17, S 12.06, Found C 58.70, H 3.94, N 13.18, S 12.09.

3,5-Dimethyl-*N*²,*N*⁴-bis(4-(4-nitrophenyl)thiazol-2-yl)-1*H*-pyrrole-2,4-dicarbox amide (**3d**)

IR (cm⁻¹): 3351.32 (NH), 3027.30 (Ph-CHstr), 2753.20 (CH₃), 1682.10 (OCNH), 1527 (NO₂), 1512 (C=N in thiazole ring), 619.09 (C-S-C); ¹H NMR (DMSO-*d*₆): 12.92 (s,1H, OCNH), 11.57 (s,1H, NH), 7.69 (s, 1H, CH in thiazole ring), 7.31-7.52 (m,5H, Ph-H), 2.38 (s, 3H, C3-CH₃), 2.10 (s, 3H, C5-CH₃); ¹³C NMR (CDCl₃): 168.30 (CONH), 166.54 (NH-C), 151.79 (Ph-C in thiazole), 144.60 (C3, C5-CH₃), 141.32-128.21 (Ph), 103.77 (CH-Thiazole), 14.23 (C5-CH₃), 10.91 (C3-CH₃). EI-MS, *m/z* (M⁺, Relative intensity %): 589.59 (M⁺, 14%), 499.28, 347.41, 333.38, 319.36 (100%), 221.23, 85.10. Elemental Analysis: C₂₆H₁₉N₇O₆S₂, Calc. C 52.96, H 3.25, N 16.63, S 10.88, Found C 52.93, H 3.24, N 16.60, S 10.84.

*N*²,*N*⁴-bis(4-(4-methoxyphenyl)thiazol-2-yl)-3,5-dimethyl-1*H*-pyrrole-2,4-dicarbox amide (**3e**)

IR (cm⁻¹): 3326.33 (NH), 3026.23 (Ph-CHstr), 2750.31 (CH₃), 1676.95 (OCNH), 1513.44 (C=N in thiazole ring), 617.10 (C-S-C); ¹H NMR (DMSO-*d*₆): 12.90 (s,1H, OCNH), 11.51 (s,1H, NH), 7.76 (s,1H, CH in thiazole ring), 7.11-7.32 (m,5H, Ph-H), 2.41 (s, 3H, C3-CH₃), 2.23 (s, 3H, C5-CH₃); ¹³C NMR (CDCl₃): 168.56 (CONH), 166.60 (NH-C), 151.81 (Ph-C in thiazole), 144.02 (C3, C5-CH₃), 161.32 (C-OCH₃), 142.31-128.41 (Ph), 103.80 (CH-thiazole), 55.45 (C-OCH₃), 14.16

(C5-CH₃), 10.94 (C3-CH₃). EI-MS, *m/z* (M⁺, Relative intensity %): 559.81(M⁺, 83%), 499.01, 423.51, 151.16 (100%), 123.15, 95.14. Elemental Analysis: C₂₈H₂₅N₅O₄S₂, Calc. C 60.09, H 4.50, N 12.51, S 11.46, Found C 60.10, H 4.54, N 12.53, S 11.49.

Biological evaluation

In vitro antibacterial screening

The compounds **2** and **3a-e** were evaluated for their *in vitro* antibacterial activity against *Escherichia coli* (MTCC-739) and *Staphylococcus aureus* (MTCC-96), by disc diffusion method [12]. The bioassay was performed using Mueller-Hinton agar (Hi-Media) medium. Ciprofloxacin was used as a reference drug. Each compound was tested at concentration 100µg/mL in DMSO. The zone of inhibition (mm) was measured after 24h incubation at 37°C.

In vitro antifungal screening

The compounds **2**, **3a-e** were evaluated for their *in vitro* antifungal activity such as *Aspergillus niger*, *Candida albicans* (recultured) using disc diffusion method [13] with sabouraud's dextrose agar (Hi-Media). Clotrimazole was used as a reference drug. Each compound was tested at a concentration of 100µg/mL in DMSO. The zone of inhibition (mm) was measured incubated at 37°C.

Acknowledgements

We wish to thank, Department of Microbiology Bharathidasan University, for their help in antimicrobial activities. We sincerely thank to management of Jamal Mohamed College, for providing laboratory facilities.

References

1. Almerico, A. M.; Diana, P.; Barraja, P.; Dattolo, G.; Mingoia, F.; Loi, A.G.; Scintu, F.; Milia, C.; Puddu, I.; La Colla, P. *Il Farmaco* **1998**, 53, 33-40.
2. Carpio, H.; Galeazzi, E.; Greenhouse, R.; Guzman, A.; Velarde, E.; Antonio, Y.; Franco, F.; Leon, A.; Perez, V.; Salas, R.; Valdes, D.; Ackrell, J.; Cho, D.; Gallegra, P.; Halpern, O.; Koehler, R.; Maddox, M.L.; Muchowski, J.M.; Prince, A.; Tegg, D.; Thurber, T.C.; VanHorn, A. R.; Wren, D. *Can. J. Chem.* **1982**, 60, 2295-2312.
3. Dannhar, D.T.; Kiefer, G.; Kramer, W.; Maehrlin, G.; Nowe, S.; Fiebich, U. *Eur. J. Med. Chem.* **2000**, 35, 499-510.
4. Evans, M.A.; Smith, D.C.; Holub, J.M.; Argenti, A.; Hoff, M.; Dalglish, G.A.; Wilson, D.L.; Taylor, B.M.; Berkowitz, J.D.; Burnham, B.S.; Krumpe, K.; Gupton, J.T.; Scarlett, T.C.; Durham, R.; Hall, I.H. *Arch. Pharm. Pharm. Med. Chem.* **2003**, 336, 181-190.
5. Geronikaki, A.; Theophilidis, G. *Eur. J. Med. Chem.* **1992**, 27, 709-716.

6. Giridhar, T.; Reddy, R.B.; Prasanna, B.; Chandra Mouli, G.V.P. *Indian J. Chem.* **2001**, *40B*, 1279-1281.
7. King, L.C.; Hlavacek, R.J. *J. Am.Chem.Soc.* **1950**, *72*, 3722-3725.
8. Siddiqui, H.L.; Iqbal, A.; Ahmad, S.; Weaver, G.W. *Molecules* **2006**, *11*, 206-211.
9. Fischer, H.; Nöller, C. R. *Org. Synth.* **1943**, *15*, 17
10. Fadda, A. A.; Bondock, S.; Rabie, R.; Etman, H. A. *Turk. J. Chem.* **2008**, *32*, 259-286.
11. Refaat, H. M.; Moneer, A. A.; Khalil, O. M. *Arch. Pharm. Res.* **2004**, *27*, 1093-1098.
12. Bauer, A. W.; Kirby, W. M.; Sherris, J. C. *Am. J. Clin. Pathol.* **1966**, *39*, 493-496.
13. Varma, R. S. Editor, *Antifungal Agents: Past, Present and Future prospects*, National Academy of Chemistry and Biology, Lucknow, India, **1998**.