Research Article

Design, synthesis and antifungal activity of 4-[2,4,5-tri(4-chlorophenyl)-1*H*-imidazol-1-yl]butanoic acid

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Abstract
A new imidazole derivative was designed, synthesized and screened for its antifungal activity. The results showed that the synthesized compound **7** exhibited antifungal activity against Aspergillus flavus but no activity against Candida albicans. The inhibition zone at 10 mg/ml was around 14 mm while the inhibition zone for the ketoconazole was 16 mm..
Keywords: Imidazole, Antifungal Activity

Introduction
In recent years, systemic fungal infections have become increasingly common, especially in the immunocompromised hosts with cancer or AIDS and in organ transplant cases[1,2]. Among the antifungal agents, azoles were used widely in treatment of fungal infections[3].

Antifungal agents, such as fluconazole, voriconazole and bifonazole have been approved (**Fig. 1**) and used ass potent antifungal agents[4]. However, these antifungal agents exist many problems, such as drug resistance, narrow antifungal spectrum and low bioavailability, which negatively affect their clinical efficacy[5, 6]. Therefore, it is necessary to develop novel antifungal compounds with potent activity, broad spectrum and low resistance.

The imidazoles are one of the two major classes of antifungal azole derivatives. Recently, newer antifungal imidazole derivatives have been developed such as 5-nitro-1*H*-imidazolde derivative compound **I** and 5-bromo-2-styryl benzimidazole derivative compound **II**, which have been reported to exhibit potent anti-fungal activity against Candida albicans and Aspergillus fumigates[7, 8]. In this line, we have designed, synthesized and also evaluate the antifungal activity of a new imidazole derivative.

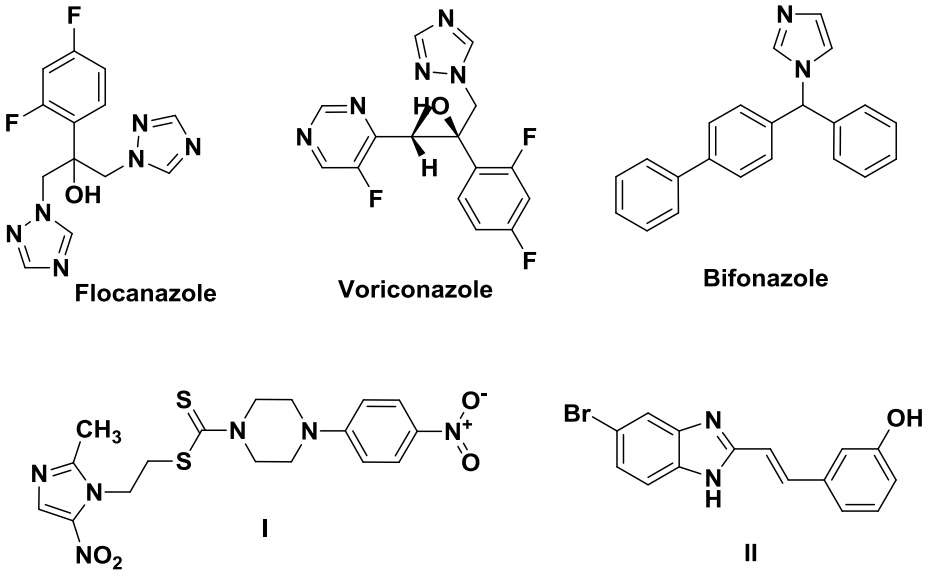


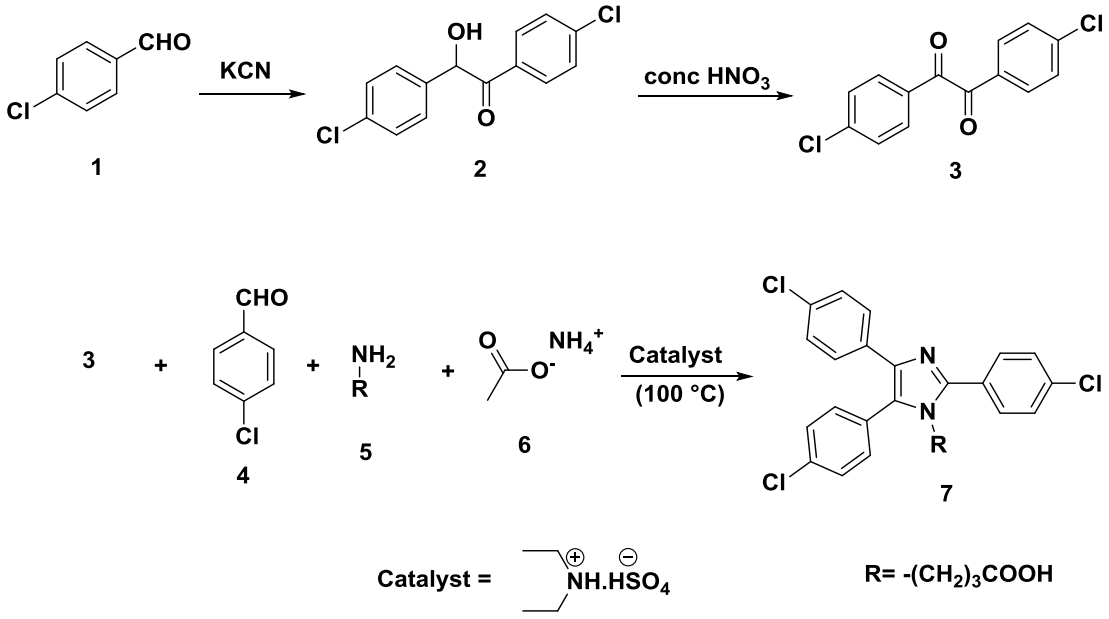
Fig. 1. Chemical structures of azole antifungal agents and compounds **I** and **II**.

Results and discussion

Chemistry

Compound **3** was synthesized through reaction of 4-chlorobenzaldehyde with ethanol in the presence of KCN as catalyst. The 4,4'-dichloro benzoin derivative is obtained after refluxing and subjected to steam distillation for 1 hour. The crude 4,4'-dichloro benzoin obtained was then refluxed with conc. HNO_3 for 1 hour to produce compound **3**.

Multi-component reaction was done through condensation of compound **3** with 4-chloroaldehyde **4**, 4-amino butyric acid **5** and ammonium acetate **6** using diethyl ammonium hydrogen sulphate, and ionic liquid catalyst under solvent free conditions afforded the target compound **7**[9].



Antifungal evaluation

The synthesized compound **7** was screened for its antifungal activity against *Aspergillus flavus* (RCMB 002002) and Candida albicans RCMB 005003 (1) ATCC 10231using ketoconazole as standard drug. The results showed that the newly synthesized compound **7** exhibited antifungal activity against *Asp. flavus* but no activity against Candida albicans. The inhibition zone at 10 mg/ml was around 14 mm while the inhibition zone for the control drug was 16 mm.

Conclusion

Compound **7** exhibited promising antifungal activity against Aspergillus flavus compared to the reference drug ketoconazole.

Experimental

Chemistry

Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. Reactions were monitored by TLC (Kieselgel 60 F254 precoated plates, E. Merck, Germany), the spots were detected by exposure to UV lamp at λ 254nm. Melting points were determined on an electro thermal melting point apparatus (Stuart Scientific Co.) and were uncorrected. ^{1}HNMR spectrum was determined in DMSO-d_6. NMR instruments BRUKER Avance III 400 MHz NMR spectrometer, Faculty of Science, Cairo University, Egypt. Chemical shifts δ are reported in parts per million (ppm) downfield from tetramethylsilane (TMS) internal standard. Elemental microanalyses were performed at the Regional Center for Mycology and Biotechnology, Al-Azhar University, Egypt.

Preparation and analytical data of compound **3** was as reported [10].

Synthesis of 4-[2,4,5-tri(4-chlorophenyl)-1*H*-imidazol-1-yl]butanoic acid 7

To 1,2-bis(4-chlorophenyl)ethane-1,2-dione (5 mmol, 1.395g) in rounded flask 50 mL, add equivalent amount of 4-chlorobenzaldhyde (5

mmol, 0.703 g), ammonium acetate (5 mmol, 0.385 g), 4-amino butyric acid (5 mmol, 0.516 g) and diethyl ammonium hydrogen sulfate (3 mmol, 0.510 g). The mixture warmed without stirring at 70-80°C till all flask component have melted, then begin stirring at 100 °C for 30 min. The reaction mixture is then triturated with water and the resulting precipitate was filtered, washed with water and dried. Recrystallization was done from aqueous ethanol.

Brown crystals m.p. 93-95 °C (yield 85%); ^{1}H NMR (400 MHz, DMSO) δ 12.07 (s, 1H, OH), 8.13 – 7.21 (m, 12H, Ar-H), 3.97 (t, *J* = 7.2 Hz, 2H, CH_2), 1.92 (t, J = 7.0 Hz, 2H, CH_2), 1.49 (m, 2H, CH_2-$\underline{CH_2}$-CH_2). Elemental analysis for $C_{25}H_{19}C_{l3}N_2O_2$ (Calcd./Found): C, 61.81/62.08; H, 3.94/3.90; N, 5.77/5.94

Antifungal screening

The Susceptibility test was performed according to CLSI recommendations (Clinical Laboratory Standard Institute, 2016). Screening test regarding the inhibition zone was carried out for compound **7** by well diffusion method[11]. The inoculum suspension for each test organism was prepared from colonies grown over night on an agar plate. It was inoculated into malt broth. A sterile swab was immersed in the microbial suspension in which the turbidity was adjusted to McFarland 0.5. The microbial suspension was inoculated malt agar plates. The compound was dissolved in dimethyl sulfoxide (DMSO) and 10mg/ml was used as the screening concentration. The inhibition zone for compound was measured in mm around each well after 24hrs at 37C^o inoculation controls using DMSO was adequately done. Ketoconazole as antifungal is used as control reference.

References

1. S.K. Fridkin, W.R. Jarvis, Epidemiology of nosocomial fungal infections, Clin. Microbiol. Rev., 9 (1996) 499-511.
2. J.R. Wingard, H. Leather, A new era of antifungal therapy, Biol. Blood Marrow Transplant., 10 (2004) 73-90.
3. M.M. Canuto, F.G. Rodero, Antifungal drug resistance to azoles and polyenes, The Lancet infectious diseases, 2(2002)550-563.
4. D. Allen, D. Wilson, R. Drew, J. Perfect, Azole antifungals: 35 years of invasive fungal infection management, Expert Rev. Anti Infect. Ther., 13 (2015) 787-798.
5. A. Lupetti, R. Danesi, M. Campa, M. Del Tacca, S. Kelly, Molecular basis of resistance to azole antifungals, Trends Mol. Med., 8 (2002) 76-81.
6. P. Kale, L.B. Johnson, Second-generation azole antifungal agents, Drugs of today, 41 (2005) 91-106.
7. R.V. Shingalapur, K.M. Hosamani, R.S. Keri, Synthesis and evaluation of in vitro anti-microbial and anti-tubercular activity of 2-styryl benzimidazoles, Eur. J. Med. Chem., 44 (2009) 4244-4248.
8. L. Kumar, A. Sarswat, N. Lal, V.L. Sharma, A. Jain, R. Kumar, V. Verma, J.P. Maikhuri, A. Kumar, P.K. Shukla, Imidazole derivatives as possible microbicides with dual protection, Eur. J. Med. Chem., 45 (2010) 817-824.
9. S.K. Mohamed, J. Simpson, A.A. Marzouk, A.H. Talybov, A.A. Abdelhamid, Y.A. Abdullayev, V.M. Abbasov, Multicomponent green synthesis, spectroscopic and structural investigation of multi-substituted imidazoles. Part 1, Zeitschrift für Naturforschung B, 70 (2015) 809-817.
10. M. Mousavi, H. Seyfi, One-pot Synthesis of Benzil Derivatives from Aromatic Aldehydes.
11. J. Hindler, B. Howard, J. Keiser, Antimicrobial agents and antimicrobial susceptibility testing, Howard BJ. Clinical and Pathogenic Microbiology. 2nd ed. St. Louis: Mosby, (1994).