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Synthesis of Novel Highly Potent Antibacterial and Antifungal Agents

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ABSTRACT: A series of tetrasubstituted imidazoles were synthesized. Compounds were synthesized through a rapid one-pot reaction via microwave irradiation. All the synthesized compounds were characterized by ^{1}H-NMR, IR and Mass analysis and screened them for their antimicrobial activity against Gram positive, Gram negative and fungal species. Antibacterial activity of all the synthesized compounds was determined by the disc diffusion method on nutrient agar medium. The sterile medium (Nutrient Agar medium (NA, 15 ml) in each petriplate was uniformly smeared with cultures of *Salmonella typhi*, *Bacillus subtilis* (both Gram+ve), *Staphylococcus aureus*, *Xanthomonas oryzae* and *Escherichia coli* (all Gram–ve). Potato Dextrose Agar (PDA) media was prepared and about 15 ml of the medium was poured into each petriplate and allowed to solidify. Five mm disc of 7-day-old culture of the test fungi (*Fusarium oxysporum* and *Aspergillus niger*) were placed at the center of the petriplates and incubated at 25±2 ºC for seven days.
KEYWORDS: Antibacterial; Antifungal; Imidazole.

ORIGINAL ARTICLE

INTRODUCION

The incidence of invasive microbial infections caused by opportunistic pathogens, often characterized by high mortality rates, has been increasing over the past two decades. Patients who become severely immune-compromised because of underlying diseases such as leukemia or recently acquired immunodeficiency syndrome or patients who undergo cancer chemotherapy or organ transplantation are particularly susceptible to opportunistic microbial infection (Goker et al., 2002). Almost all of the major classes of antibiotics have encountered resistance in clinical applications. The emergence of bacterial resistance to B-lactam antibiotics, macrolides, quinolones and vancomycin is becoming a major worldwide health problem (He et al., 2003). A matter of concern in the treatment of microbial infections is the limited number of efficacious antimicrobial drugs. Many of the currently available drugs are toxic, enable recurrence because they are bacteriostatic/fungistatic and not bactericidal/fungicidal or lead to the development of resistance due in part to the prolonged periods of administration (Fostel and Lartey, 2000). There is a real perceived need for the discovery of new compounds that are endowed with antibacterial and antifungal activities, possibly acting through mechanism of actions, which are distinct from those of well-known classes of antimicrobial agents to which many clinically relevant pathogens are now resistant (Khalafi-Nezhad et al., 2005). The herpes virus family contains eight known human viruses; amongst them are herpes simplex virus-1 (HSV-1) and herpes, simplex virus-2 (HSV-2) that cause mucocutaneous infections resulting in cold sores (HSV-1) and genital lesions (HSV-2), respectively. Much research has been focused on HSV-1 and HSV-2 as these viruses have a high incidence rate and a high prevalence (Gudmundsson et al., 2008). Vesicular stomatitis virus (VSV), a member of the Rhabdoviridae family, is an enveloped single-stranded RNA virus that causes an economically important disease in cattle, horses and swine (Romanutti et al., 2007). The alphaviruses are a genus of approximately 27 arthropodtransmitted plus-strand RNA viruses found in the Togaviridae family which is responsible for a wide range of diseases and many of them are important human and animal pathogens. Several examples of alphaviruses are Sindbis, Semliki Forest, and Venezuelan equine encephalitis viruses. Infection can result in fever, rash, arthralgia or arthritis, lassitude, headache, and myalgia. The prototypic alphavirus is Sindbis virus (SINV), which is transmitted to humans through mosquito bites (Kim et al., 2007). Respiratory syncytial virus (RSV), a paramyxovirus, is an important cause of respiratory tract infection in infants, young children and adults (Andries et al., 2003). Previous antiviral research on herpes simplex viruses has primarily focused on the development of nucleoside analogues, such as acyclovir (Zovirax), valacyclovir, famciclovir, and penciclovir. Recently, immunomodulators (imiquimod and resiquimod), nonnucleoside viral polymerase inhibitors (4-hydroxyquinoline-3- carboxamides), and viral helicase inhibitors (thiazolylphenyl and thiazolylamide) have received considerable attention. Though numerous strategies and considerable efforts have been spent in search of the next generation antiviral therapy, it has proved difficult to outperform acyclovir (Gudmundsson et al., 2008). The incorporation of the imidazole nucleus is

an important synthetic strategy in drug discovery. The high therapeutic properties of the imidazole related drugs have encouraged the medicinal chemists to synthesize a large number of novel chemotherapeutic agents. Imidazole drugs have broadened scope in remedying various dispositions in clinical medicines. Medicinal properties include anticancer (Congiu et al., 2008), B-lactamase inhibitors (Venkatesan et al., 2008), 20-HETE synthase inhibitors (Nakamura et al., 2004), carboxypeptidase inhibitors (Han and Kim, 2001), hemeoxygenase inhibitors (Roman et al., 2007), antiaging agents (Babizhayev et al., 2004), anticoagulants (Nantermet et al., 2005), anti-inflammatory (Adams et al., 2001), antibacterial (Bhandari et al., 2009), antifungal (Emami et al., 2008), antiviral (Ujjinamatada et al., 2007), antitubercular (Zampieri et al., 2008), antidiabetic (Crane et al., 2006) and antimalarial (Valhhakis et al., 2006), all are unique characteristics known for imidazole derivatives. In the present study we have synthesized tetrasubstituted imidazoles (5a-f) and screen them for their antibacterial and antifungal activity.

MATERIALS AND METHODS

Chemicals and reagents

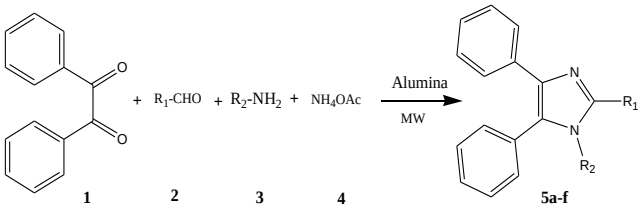
All the chemicals and solvents used for this work were obtained from E-Merck Ltd., and S.D. Fine Chem. Ltd., Mumbai. Acidic ϒ -Alumina (surface area 22 m^2/g) were provided by National Chemical Laboratory, Pune (India) and were used as such in the synthesis of Benzimidazole Derivatives.

Characterization

Microwave system (OM 9925-E, 230V—50Hz) of Kenstar make (Mumbai) was used and the output of microwave power is mentioned as percent intensity *i.e.* 20%, 40%, 60%, 100%. Melting points of the synthesized compounds were determined in open capillary tubes and were uncorrected. IR absorption spectra were recorded on Jasco FTIR-4100 series instrument, KBr diffuse reflectance, ^{1}H-NMR spectra were recorded on a Shimadzu AMX 400-Bruker 400-MHz spectrometer using DMSO-d_6 as solvent and TMS (tetramethylsilane) as an internal standard. The ^{1}H chemical shifts were reported as parts per million (ppm) downfield from TMS (Me_4Si). Mass spectra were determined in an ionization energy (EI) at 70 eV ionizing voltage. ^{1}H-NMR and IR spectra were consistent with the assigned structures. Purity of the compounds was checked by thin layer chromatography (TLC).

Synthesis of new Tetrasubstituted Imidazoles using benzaldehyde derivatives and primary amine

Synthesis of tetra substituted imidazole derivatives is outlined in Figure 1. Benzil (421 mg, 2 mmol), benzaldehyde derivatives (2 mmol), ammonium acetate (2 mmol), primary amine (2 mmol) and 100 mg of Alumina were mixed thoroughly in a mortar. The reaction mixture was then irradiated in a domestic microwave oven for 20 min (optimized time) at 160 W. The progress of reaction was monitored by TLC using n-Hexane: Ethyl acetate (90:10) as eluent. The mixture was extracted with chloroform, and the solvent was removed by rotary evaporation. Further purification by column chromatography and recrystallization gave the desired products (5a-f).



5a:R_1 = 3,4,5-$(OCH_3)_3C_6H_2$, R_2 = 4-ClC_6H_4
5b:R_1 = 3,4,5-$(OCH_3)_3C_6H_2$, R_2 = 3-Cl,4-FC_6H_3
5c:R_1 = 3-OH,4-$OCH_3C_6H_3$, R_2 = 4-ClC_6H_4
5d:R_1 = 3-OH,4-$OCH_3C_6H_3$, R_2 = 3-Cl,4-FC_6H_3
5e:R_1 = 4-ClC_6H_4 , R_2 = 4-ClC_6H_4
5f:R_1 = 4-$OCH_3C_6H_4$, R_2 = 4-ClC_6H_4

MW = Microwave Irradiation

Figure 1. Synthesis of Tetrasubstituted Imidazoles

Products characterization data of Novel Compounds (5a-f)

5a: M.p.: 237–239 °C; IR ν_{max} (KBr, cm^{-1}): 2950 (C–H), 1600 (C=C), 1580 (C=N), 1505; ^{1}H NMR (DMSO-d_6) δ: 3.45-3.85 (s, 9 H, OCH_3), 6.90–7.80 (m, 16 H, Ph) ppm; MS (m/z): 496.8.

5b: M.p.: 241–243 °C; IR ν_{max} (KBr, cm^{-1}): 2950 (C–H), 1600 (C=C), 1580 (C=N), 1505; ^{1}H NMR (DMSO-d_6) δ: 3.40-3.90 (s, 9 H, OCH_3), 7.10–7.80 (m, 15 H, Ph) ppm; MS (m/z): 515.1.

5c: M.p.: 284-286 °C; IR ν_{max} (KBr, cm^{-1}): 3150 (O-H), 1610 (C=C), 1575 (C=N), 1385 (C-O); ^{1}H NMR (DMSO-d_6) δ: 3.80 (s, 3H, OCH_3), 6.85-7.70 (m, 17H, Ph), 12.20 (s, 1H, OH) ppm; MS (m/z): 453.3.

5d: M.p.: 297-299 °C; IR ν_{max} (KBr, cm^{-1}): 3150 (O-H), 1610 (C=C), 1575 (C=N), 1385 (C-O); ^{1}H NMR (DMSO-d_6) δ: 3.80 (s, 3H, OCH_3), 6.90-7.70 (m, 16H, Ph), 12.20 (s, 1H, OH) ppm; MS (m/z): 470.9.

5e: M.p.: 242-244 °C; IR ν_{max} (KBr, cm^{-1}): 1610 (C=C), 1575 (C=N), 1385 (C-O); ^{1}H NMR (DMSO-d_6) δ: 6.80-7.70 (m, 18H, Ph) ppm; MS (m/z): 441.2.

5f: M.p.: 284-286 °C; IR ν_{max} (KBr, cm^{-1}): 1610 (C=C), 1575 (C=N), 1385 (C-O); ^{1}H NMR (DMSO-d_6) δ: 3.80 (s, 3H, OCH_3), 6.90-7.60 (m, 18H, Ph) ppm; MS (m/z): 437.2.

Antibacterial activity

Antibacterial activity of all the synthesized compounds was determined by the disc diffusion method on nutrient agar medium. The sterile medium (Nutrient

Agar medium (NA) 15 ml) in each petriplate was uniformly smeared with cultures of *Salmonella typhi*, *Bacillus subtilis* (both Gram +ve), *Staphylococcus aureus*, *Xanthomonas oryzae* and *Escherichia coli* (all Gram –ve). Sterile discs (10 mm diameter) were placed in each of the petriplate, to which 50µl of the different synthesized compounds were added.

The treatments also included Streptomycin as positive control for comparison. For each treatment, 4 replicates were maintained. The plates were incubated at 25±2 ºC for 24 h and the size of the resulting zone of inhibition, if any, was determined. Zone of inhibition for forty two imidazole derivatives was done and given in Table 1.

Antifungal activity

Potato Dextrose Agar (PDA) media was prepared and about 15 ml of the medium was poured into each petriplate and allowed to solidify. Five mm disc of 7-day-old culture of the test fungi (*Fusarium oxysporum* and *Aspergillus niger*) were placed at the center of the petriplates and incubated at 25±2 ºC for seven days. After incubation the colony diameter was measured in millimeter. For each treatment four replicates were maintained. PDA medium without the novel compound but with Nystatin served as positive control.

All the synthesized compounds were tested (at the dosage of 100µl of the synthesized compound/petriplate where the synthesized compound concentration was 0.1mg/ml) for antifungal activity by poisoned food technique. Zone of inhibition for forty two imidazole derivatives was done and given in Table 2.

Table 1. Antibacterial activities of synthesized compounds as inhibitory zone diameter (mm)

Compound	Inhibition zone diameter (in millimeter) against the microorganism				
	Staphylococcus Aureus	***Escherichia Coli***	***Xanthomonas Oryzae***	***Salmonella Typhi***	***Bacillus Subtillis***
5a	25	18	25	25	20
5b	24	19	21	22	7
5c	23	18	24	26	7
5d	27	18	27	23	7
5e	25	19	27	25	7
5f	24	17	22	24	6
+ Control	30	20	30	30	8

For bacteria + control: Streptomycin; For antibacterial activity disc diffusion method was used.; For antibacterial activity the concentration of the compound and for the + control: 50 µl was taken.

Table 2. Antifungal activities of synthesized compounds as inhibitory zone diameter (mm)

Compound	Inhibition zone diameter (in millimeter) against the microorganism		
	Fusarium oxysporum	***Aspergillus niger***	***Aspergillus fumiigatus***
5a	85	84	90
5b	68	82	88
5c	65	80	86
5d	66	88	84
5e	67	85	88
5f	66	83	89
+ Control	70	90	90

For fungus + control: Nystatin; For antifungal activity pour plate method was used; For antifungal activity the concentration of the compound and for the + control: 100µl was taken.

RESULTS

A series of tetrasubstituted imidazoles were synthesized. Compounds were synthesized through a rapid one-pot reaction via microwave irradiation. All the synthesized compounds were characterized by ^{1}H-NMR, IR and Mass analysis and screened them for their antimicrobial activity against Gram positive, Gram negative and fungal species. Antibacterial activity of all the synthesized compounds was determined by the disc diffusion method on nutrient agar medium. The sterile medium (Nutrient Agar medium (NA) 15 ml) in each petriplate was uniformly smeared with cultures of *Salmonella typhi*, *Bacillus subtilis* (both Gram +ve), *Staphylococcus aureus*, *Xanthomonas oryzae* and *Escherichia coli* (all Gram –ve) by using Streptomycin as positive control. Potato Dextrose Agar (PDA) media was prepared and about 15 ml of the medium was poured into each petriplate and allowed to solidify. Five mm disc of 7-day-old culture of the test fungi (*Fusarium oxysporum* and *Aspergillus niger*) were placed at the center of the petriplates and incubated at 25±2 ºC for seven days by using Nystatin as a positive control.

According to the tables and as mentioned above altogether among the synthesized compounds were

studied, demonstrated potent inhibition against all the strains tested and they have similar activity to the positive control compounds Streptomycin and Nystatin respectively. The compounds described in Tables 1 and 2 are tetrasubstituted imidazole derivatives having different functional groups like fluoro, chloro, hydroxyl and methoxy**.**

DISCUSSION

The compound, are tetrasubstituted imidazole derivatives having different functional groups like halogens (Fluorine and Chlorine), hydroxyl and methoxy groups. According to what presented in Table 1, compound 5a was found to possess high antibacterial activity against *Bacillus subtilis*, it showed more antibacterial activity than the standard drug Streptomycin. The other tested compound showed less antibacterial activity than the standard drug. In the case of Table 2 same compound 5a was found to be highly antifungal potential against *Fusarium oxysporum* and activities equivalent to that of the standard drug Nystatin against *Aspergillus fumiigatus*. The novel imidazole derivatives and particularly compound 5a, with these types of functional groups have better antimicrobial activity which may be due to bonding with organism cell membrane molecules increase the inhibition.

Among the compounds studied, novel compounds (5a-f), which are *N*1-Substituted 4,5-diphenylimidazole derivatives having different functional groups like fluorine, chlorine, hydroxyl and methoxy exhibited high antibacterial and antifungal activities in comparison to *N*1-non-substituted 4,5-diphenylimidazole derivatives and 2-substituted benzimidazoles. Compound 5a was shown higher antifungal activity against *Fusarium oxysporum* and activity equivalent to that of the standard drug Nystatin against *Aspergillus fumiigatus* but other compounds were shown less activity than the standard drug against the same fungi. In the case of antibacterial activity, the same compound 5a was found to be highly active against *Bacillus subtilis* but other compounds were shown less activity than the standard drug Streptomycin against the same bacteria. These better antibacterial and antifungal activities may be due to the presence of functional groups bonding with organism cell membrane molecules increases the inhibition.

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