Available online at www.cpronline.in

Current Pharma Research 3 (4), 2013, 989–993

Current Pharma Research

http://www.cpronline.in

Original Article

Synthesis and Evaluation of 2, 4, 5 - Trisubstituted and 1, 2, 4, 5 Tetrasubstituted Imidazole as Antimicrobial Agents.

M. G. Kanawade [a], P. S. Dabhade[a,*], A. S. Dange[a]

[a]*S.N.D. College of Pharmacy, Babhulgaon, Yeola, Nashik - 423401, Maharashtra, India*

Received 4 July 2013; received in revised form 18 July 2013; accepted 19 July 2013

Available online 15 September 2013

Abstract

A series of 2,4,5-trisubstituted (A1-A15) and 1,2,4,5-tetrasubstituted (B1-B7) imidazole derivatives were synthesized by the reaction of Benzil, aldehyde, ammonium acetate, aromatic primary amines using catalyst tetrabutylammoniumbromide in isopropanol. Structures of these compounds were elucidated by spectral (IR, ^{1}H NMR) analysis. The newly synthesized compounds were screened for antifungal and antibacterial activities. The activity was measured in terms of zone of inhibition and compared with standard drug. Compounds A4,A7,A8,A10,A12,A14,A15,B1,B3,B5 and B6 have shown good antibacterial activity against std. Norfloxacin and Compound A2,A3,A6,A7,A9,A10,A14,A15,B2,B3,B4,B6 and B7 exhibited good antifungal activities against std. Clotrimazole, and other compound shows moderate activity.

Keywords: *Imidazole, Antibacterial activity; Antifungal activity.*

1. Introduction

In the past decades, fungal infections have increased, especially in immuno-compromised patients [1–4]. Candidiasis caused by *Candida albicans* is also one of the most frequent (though uncommonly life threatening) fungal infections attacking persons with HIV/ AIDS [5]. On the other hand, development of resistance, particularly with suppressed immunity, is a challenging problem in the treatment of fungal infections [6].Substituted imidazole represents a class of heterocyclic analogues having valuable pharma-cological properties such as antiparasitic [7], antifungal [8], antimicrobial [9-13], and antidepressant [14] activity. In addition, many of the substituted diaryl imidazoles are known as potential inhibitors of the p38 MAP kinase [15].The development of new and different antimicrobial agents has been an important step.The infection caused by *staphylococcus aures,enterococci* and *pneumococci* are particularly problematic [16]. There is need for the discovery of new compounds,possibly acting through mechanism of action that are distinct from those of well-known classes of antimicrobial agents to which many clinically relevant pathogen are now resistant [17].

2. Material and Methods

All chemicals were of analytical grade and were used directly. All melting point were determined in open capillary method and were uncorrected. Infrared spectra were recorded on Bruker Software-OPUS, Model-ALPHA spectrometer, ^{1}H NMR spectra were acquired on the Bruker Avance-2 model spectrophotometer using Chloroform as a solvent and TMS as internal reference (chemical shift in δ, ppm).

All products were purified by recrystallisation. The reaction were followed up and the purity of product was carried out on TLC plates (Silica Gel), visualizing the spots under Iodine fumes.

2.1. General procedure for the synthesis of 2, 4, 5 trisubstituted imidazoles [18]

To a stirred mixture of benzil (2.5 mmol) and tetrabutylammonium bromide (0.08 g, 0.25 mmol, 10 mol%) in isopropanol (5 mL), substituted aldehyde (2.5 mmol) and ammonium acetate (25 mmol) were added at room temperature and the reaction mixture was heated to 82^{o}C with stirring for 15–30 min by refluxing. After completion of reaction (monitored by TLC) the mixture was cooled to 10^{o}C, the resulting solid was filtered. The products obtained were pure (single spot on TLC). They were further purified by recrystallization and were fully characterized by spectroscopic methods such as IR, ^{1}H NMR.

2.1.1. 2-(Phenyl)-4,5-diphenylimidazole ***(A1)***

IR (cm-^{1}) - 3236.49(N-H), 3066.81(C-H), 1651.54(C=N), 1270.66(C-N), 1556.01(C=C).

2.1.2. 2-(4-nitrophenyl) -4,5-diphenylimidazole ***(A2)***

IR (cm-^{1}) - 3210.10(N-H), 3058.34(C-H), 1653.03(C=N), 1560.45(C=C), 1403.60(N=O), 1339.23(C-N).

2.1.3. 2-(2,6-dichloroPhenyl)-4,5-diphenylimidazole ***(A3).***

IR (cm-^{1}) - 3278.65(N-H), 3088.10(C-H), 1656.06(C=N), 1490.67(C=C), 1270.97(C-N), 714.90(C-Cl). ^{1}H NMR (400MH$_Z$): δ [7.22-

*Corresponding author.
E-mail address: pratap.dabhade@gmail.com (P.S.Dabhade)

7.48,m,(10H) Ar H],[13.4(1H)NH], [7.21,s,(2H)Ar-H], [7.10,s,(1H) Ar-H].

2.1.4. 2-(4-BromoPhenyl)-4,5-diphenylimidazole ***(A4)***.
IR (cm-^{1}) - 3231.98(N-H), 3089.10(C-H), 1652.01(C=N), 1550.10(C=C), 1287.45(C-N), 640(C-Br).

2.1.5. 2-(2-HydroxyPhenyl)-4,5-diphenylimidazole ***(A5)***.
IR (cm.-^{1}) - 3389.03(N-H), 3218.22(-OH), 3059.09(C-H), 1646.17(C=N), 1528.78(C=C), 1312.33(C-N).

2.1.6. 2-(4-trifluoromethylPhenyl)-4,5-diphenylimidazole ***(A6).***
IR (cm-^{1}) - 3375.67(N-H), 3085.02(C-H), 1642.96(C=N), 1590.06(C=C), 1324.56(C-N), 1120.45(trifluoromethyl).

2.1.7. 2-(2-trifluoromethylPhenyl)-4,5-diphenylimidazole ***(A7).***
IR (cm-^{1}) - 3163.10(N-H), 3072.28(C-H), 1654.03(C=N), 1490.34(C=C), 1309.91(C-N), 1127.65(trifluoromethyl). ^{1}H NMR (400MH$_Z$): δ [7.22-7.48,m,(10H)],[13.4(1H) NH],[7.51,s,(1H)Ar-H], [7.15,s,(1H)Ar-H], [7.32,s,(1H) Ar-H], [7.41,s,(1H) Ar-H].

2.1.8. 2-(4-MethylthioPhenyl)-4,5-diphenylimidazole ***(A8).***
IR (cm-^{1})-3412.43(N-H), 3098.84 (C-H), 2963.32(C-H of CH3), 1645.71(C=N), 1490.45(C=C), 1346.98(C-N), 714.67(C-S). ^{1}H NMR (400MH$_Z$):δ [7.22-7.48,m,(10H)Ar-H],[13.4,(1H),NH],[7.24 7.38,s,(4H)Ar],[2.47,(3H),C-H of CH$_3$].

2.1.9. 2-(4-ChloroPhenyl)-4,5-diphenylimidazole ***(A9).***
IR (cm-^{1}) - 3210.01(N-H), 3090.12(C-H), 1656.86(C=N), 1595.01(C=C), 1290.09(C-N), 671.45(C-Cl).

2.1.10. 2-(2,4-dichloroPhenyl)-4,5-diphenylimidazole ***(A10).***
IR (cm-^{1}) - 3278.34(N-H), 3089.12(C-H), 1682.34(C=N), 1530.98(C=C), 1259.96(C-N), 690.22(C-Cl).

2.1.11. 2-(3-HydroxyPhenyl)-4,5-diphenylimidazole ***(A11).***
IR (cm-^{1}) - 3345.43(N-H), 3277.30(-OH), 3096(C-H), 1628.34(C=N), 1492.34(C=C), 1266.10(C-N).

2.1.12. 2-(4-HydroxyPhenyl)-4,5-diphenylimidazole ***(A12).*** IR (cm-^{1}) - 3245.70(-OH), 3176.09(N-H), 3066.09(C-H), 1609.75(C=N), 1500.09(C=C), 1346.34(C-N).

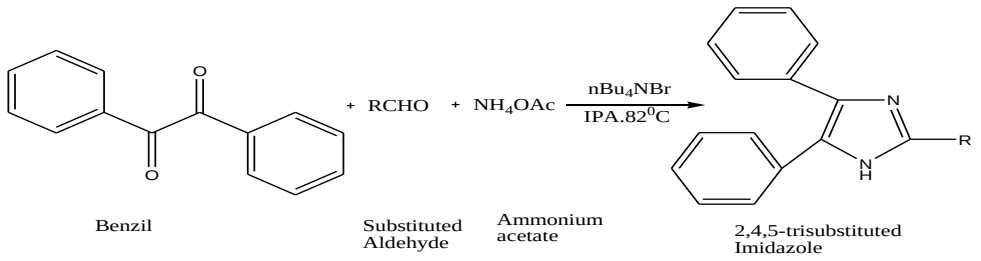
2.1.13. 2-(3-NitroPhenyl)-4,5-diphenylimidazole ***(A13).***
IR (cm-^{1}) - 3360.79(N-H), 3138.29(C-H), 1651.49(C=N), 1520.10(C=C), 1446.43(N=O), 1333.33(C-N).

2.1.14. 2-(4-DimethylaminoPhenyl)-4,5-diphenylimidazole ***(A14).***
IR (cm-^{1}) - 3424.34(N-H), 3120.02(C-H), 2969.89(C-H of CH$_3$), 1642.86(C=N), 1520.98(C=C), 1326.08(C-N), 1283.27(C-N).

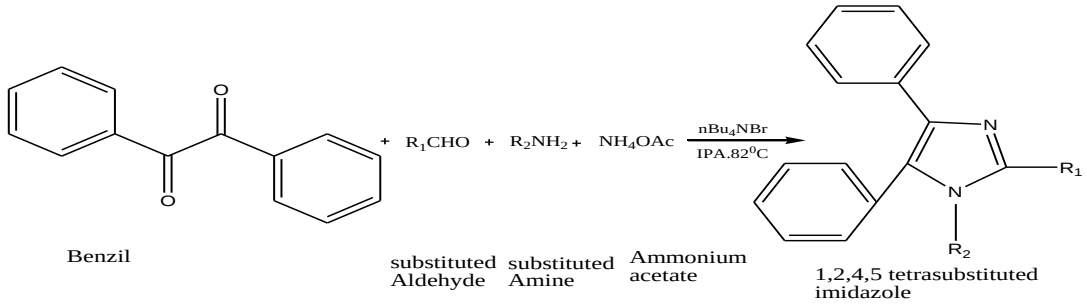
2.1.15. 2-(2-Chloroquinoline)-4,5-diphenylimidazole ***(A15).*** IR (cm-^{1}) - 3411.92(N-H), 3092.16(C-H), 1636.15(C=N), 1526.36(C=C), 1298.19(C-N), 707.63(C-Cl).

2.2 General procedure for the synthesis of 1,2,4,5 tetrasubstituted imidazoles.

To a stirred mixture of benzil (2.5mmol) and tetrabutylammoniumbromide (0.08 g, 0.25mmol, 10 mol%) in isopropanol (5mL), substituted aldehyde (2.5mmol), substituted primary amine (2.5mmol) and ammonium acetate (25mmol) were added at room temperature and the reaction mixture was heated to 82^{o}C with stirring for 15–30 min by refluxing. After completion of reaction (monitored by TLC) the mixture was cooled to 10^{o}C, the resulting solid was filtered. The products obtained were pure (single spot on TLC). They were further purified by recrystallization and were fully characterized by spectroscopic methods such as IR, ^{1}H NMR.



Scheme 1.



Scheme 2.

2.2.1. 1-Benzyl-2-(3-hydroxyphenyl)-4,5-diphenylimidazole ***(B1).***
IR (cm-^{1}) - 3260.59(-OH), 3120.67(C-H), 2934.56(C-H), 1647.54(C=N), 1494.30(C=C), 1340.98(C-N).

2.2.2. 1-(1-napthyl)-2-(4-hydroxyphenyl)-4,5-diphenylimidazole ***(B2).***
IR (cm-^{1}) - 3275.74(-OH), 3159.80(C-H), 1609.17(C=N), 1525.12(C=C), 1343.32(C-N).

2.2.3. 2-(4-chlorophenyl)-1-(1-methylphenyl)-4,5-diphenylimidazole ***(B3).***
IR (cm-^{1}) - 3145.66(C-H), 2970.45(C-H of CH_3), 1635.93(C=N), 1556.76(C=C), 1298.78(C-N), 752.49(C-Cl).

2.2.4. 2-(2,4-dichlorophenyl)-1-(1-methylphenyl)-4,5-diphenylimidazole ***(B4).***
IR (cm-^{1}) - 3061.76(C-H), 2962.23(C-H), 1651.62(C=N), 1471.50(C=C), 1288.42(C-N), 713.15(C-Cl).

2.2.5. 1-(4-nitrophenyl)- 2-(phenyl)-4,5-diphenylimidazole ***(B5).***
IR (cm-^{1}) - 3123.78(C-H), 1659.42(C=N), 1589.13(C=C), 1503.34(N=O), 1301.42(C-N).

2.2.6. 2-(4-methylthiophenyl)-1-(3-nitrophenyl)-4,5-diphenylimidazole ***(B6).***
IR (cm-^{1}) - 3063.10(C-H), 2959.22(C-H of CH_3), 1654.03(C=N), 1520.09(C=C), 1445.64(N=O), 1309.91(C-N). ^{1}H NMR (400MH$_Z$): δ[7.22-7.48,m,(10H)Ar], [7.24 7.38,s,(4H),Ar], [7.7,s,(1H),Ar], [7.5,s,(1H),Ar], [8.2,s,(1H),Ar].

2.2.7. 2-(4-dimethylaminophenyl)-1-(4-fluorophenyl)-4,5-diphenylimidazole ***(B7).***
IR (cm-^{1}) - 3059.79(C-H), 2960.20(C-H of CH_3), 1653.54(C=N), 1583.45(C=C), 1318.36(tertiary amine C-N), 1260.23(C-N), 1123.35 (trifluoromethyl).^{1}HNMR (400MH$_Z$):δ [7.22-7.48,m,(10H),Ar], [6.85-7.04,s,(4H)Ar], [2.78,(6H) C-H of CH_3], [6.49-7.26,s,(4H) Ar].

2.3. Antimicrobial activity

The synthesized compounds (A1-A15 and B1-B7) were screened for their antibacterial activity against Gram-positive bacteria (*Staphylococcus aureus* (ATCC 29737)) and Gram-negative bacteria (*Escherichia coli* (NCTC 10418)) by disc diffusion method using Nutrient agar media. And antifungal activity against *Aspergillus niger* (NCIM 596), *Candida albicans* (NCIM 3102) by disc diffusion method using Sabouraud-Dextrose agar media. Plates were observed within 20 to 24 hours and may be continued to incubate for 48 hours. Zone of inhibition of the compound discs were measured in mm for each organism and compared with the standard compound discs.

Results and Discussion

All the synthesized compounds were confirmed by spectroscopical techniques and evaluated for antimicrobial activity. The antibacterial activity against *Staphylococcus aureus, Escherichia coli* and antifungal activity against *Aspergillus niger, Candida albicans*. The substituent of the compounds with physicochemical properties 2,4,5 trisubstituted and 1,2,4,5 tetrasubstituted imidazole derivative are given in Table 1 and Table 2. These compounds showed antibacterial activity (zone of inhibition) against *Staphylococcus aureus, Escherichia coli* and antifungal activity (zone of inhibition) against *Aspergillus niger, Candida albicans*. Compounds A4, A7,A8,A10,A12,A14,A15,B1,B3,B5 and B6 have shown good antibacterial activity against std. Norfloxacin and Compound A2,A3,A6,A7,A9,A10,A14,A15,B2,B3,B4,B6 and B7 exhibited good antifungal activities against std. Clotrimazole. And the other compound shows moderate activity are given in Table 3.

Table 1
Physicochemical properties of 2,4,5 trisubstituted Imidazole.

Comp.	Ar	R_1	R_2	Molecular formula	Mol.wt g/mol	RF value	% yield	M.P (^{0}C)
B1.	Ph	3-OHC_6H_4	$C_6H_5CH_2$	$C_{28}H_{22}N_2O$	402	0.56	65.65	168-170^{0}C
B2.	Ph	4-OHC_6H_4	1-napthyl	$C_{31}H_{22}N_2O$	438	0.59	62.73	162-164^{0}C
B3.	Ph	4-ClC_6H_4	1-MeC_6H_4	$C_{28}H_{21}N_2Cl$	420	0.55	57.69	156-158^{0}C
B4.	Ph	2,4-Cl_2 C6H3	1-Me C_6H_4	$C_{28}H_{20}N_2Cl_2$	454	0.65	65.17	164-166^{0}C
B5.	Ph	C_6H_5	4-NO_2 C_6H_4	$C_{27}H_{19}N_3O_2$	417	0.48	59.22	232-234^{0}C
B6.	Ph	4-$SCH_3C_6H_4$	3-NO_2 C_6H_4	$C_{28}H_{21}N_3SO_2$	463	0.72	50	228-230^{0}C
B7.	Ph	4-$N(CH_3)_2C_6H_4$	4-F C_6H_4	$C_{29}H_{24}N_3F$	432	0.38	55.66	204-206^{0}C

Table 2
Physicochemical properties of 1,2,4,5 tetrasubstituted Imidazole.

Comp.	Ar	R	Molecular formula	Mol.wt g/mol	RF value	% yield	M.P (^{0}C)
A1.	Ph	C_6H_5	$C_{21}H_{16}N_2$	296	0.61	73.33	254-256^{0}C
A2.	Ph	4-$NO_2C_6H_4$	$C_{21}H_{15}N_3O_2$	341	0.77	80.95	264-266^{0}C
A3.	Ph	2,6-$Cl_2C_6H_3$	$C_{21}H_{14}N_2Cl_2$	364	0.24	61.11	164-166^{0}C
A4.	Ph	4-BrC_6H_4	$C_{21}H_{15}N_2Br$	374	0.35	68.47	262-264^{0}C
A5.	Ph	2-OHC_6H_4	$C_{21}H_{16}N_2O$	312	0.58	32.46	202-204^{0}C
A6.	Ph	4-CF_3 C_6H_4	$C_{22}H_{15}N_2F_3$	351	0.61	73.25	212-214^{0}C
A7.	Ph	2-CF_3 C_6H_4	$C_{22}H_{15}N_2F_3$	351	0.63	73.20	204-206^{0}C
A8.	Ph	4-SCH_3 C_6H_4	$C_{22}H_{18}N_2S$	342	0.23	65.11	196-198^{0}C
A9.	Ph	4-Cl C_6H_4	$C_{21}H_{15}N_2Cl$	330	0.81	69.13	176-178^{0}C
A10.	Ph	2,4$Cl_2C_6H_3$	$C_{21}H_{14}N_2Cl_2$	364	0.90	62.22	176-178^{0}C
A11.	Ph	3-OH C_6H_4	$C_{21}H_{16}N_2O$	312	0.51	72.72	202-204^{0}C
A12.	Ph	4-OH C_6H_4	$C_{21}H_{16}N_2O$	312	0.60	68.83	212-214^{0}C
A13.	Ph	3-NO_2 C_6H_4	$C_{21}H_{15}N_3O_2$	341	0.80	67.85	286-288^{0}C
A14.	Ph	4-$N(CH_3)_2$ C_6H_4	$C_{23}H_{21}N_3$	339	0.62	63.85	192-194^{0}C
A15.	Ph	2-Cl quinoline	$C_{24}H_{16}N_3Cl$	381	0.58	51.06	196-198^{0}C

Table 3.
Antibacterial and antifungal activity of the synthesized compounds.

Comp.code	Zone of inhibition at 100µg/ml. (in mm.)		Zone of inhibition at 100µg/ml. (in mm.)	
	E.coli	***S. aureus***	***A. niger***	***C. albicans***
A1	16	14	17	16
A2	18	16	21	23
A3	22	21	26	25
A4	21	24	18	20
A5	17	18	16	19
A6	16	19	23	24
A7	26	25	20	20
A8	24	26	18	22
A9	15	17	25	26
A10	23	26	26	23

A11	18	15	12	14
A12	22	22	12	18
A13	19	17	16	18
A14	24	22	24	26
A15	24	25	25	26
B1	23	22	16	18
B2	16	17	24	26
B3	23	26	24	23
B4	17	14	23	25
B5	24	24	18	17
B6	24	25	26	24
B7	14	16	24	26
Norfloxacin	28	26	-	-
Clotrimazole	-	-	26	27

Conclusion

The proposed compounds were screened for their antifungal, antibacterial activities. The proposed work has given out many active antifungal, antibacterial agents. Some of the compounds have showed moderate activities. These compounds with suitable modification can be explored better for their therapeutic activities in the future.

References

[1] H.B. Vanden, F. Dromer, I. Improvisi, M. Lozano-Chiu, J.H. Rex, D. Sanglard, Med Mycol. 36 (1998) 119–128.
[2] P.L. Shao, L.M. Huang, P.R. Hsueh. Int J Antimicrob Agents, 30 (2007) 487–495.
[3] J.B. Anderson. Nat Rev Microbiol, 3 (2005) 547–556.
[4] de Pauw B E, F. Meunier, Chemotherapy 45 (1999) 1–14.
[5] P.L. Fidel. Oral. Dis, 8 (2002) 69-79.
[6] M. Nucci, A.L. Colombo. Braz. J. Infect. Dis, 6 (2002) 124-128.
[7] A. Mukherjee, S. Kumar, M. Seth, A.P. Bhaduri. Ind J Chem, 28B (1989) 391-6.
[8] G. Ayhan-Kilcigil, N. Altanlar. Turk J Chem, 30 (2006) 223-8.
[9] S.M. Norman, R.D. Bennett, S.M. Poling, V.P. Maier, M.D. Nelson, Plant Physiol. 80 (1986) 122-5.
[10] O. Guven, T. Erdogan, H. Goker, S. Yidiz. Bioorg Med Chem Lett, 17 (2007) 2233-6.
[11] S. Ozden, H. Karatas, S. Yildiz, H. Goker. Arch Pharm Med Chem, 337 (2007) 556-62.
[12] H. Goker, M. Alp, S. Yildiz, Molecules 10 (2000) 1377-86.
[13] Podunavac-Kuzmanovic SO, D. Cvetkovic. J Serb Chem Soc, 75 (2007) 459-66.
[14] F.H. Hadizadeh, V. Hosseinzadeh, M. Shariaty, S. Kazemi, Iranian J Pharm Res. 1 (2008) 29-33.
[15] J.A. Murry. Drug Discov. Dev, 6 (2003) 945–965.
[16] E.F. Gale, A.M. Johnson, D. Kerridge, F. Wayman, J Gen Microbiol. 117 (1980) 535-8.
[17] D.K. Miller, E. Griffiths, J. Lenard, R.A. Firestone. J Cell Biol, 97 (1983) 1841-51.
[18] Mahankhali Venu Chary, Nallani Chakravarthula Keerthysri, Srinivasu V.N. Vupallapati. Catalysis Communications 9 (2008) 2013–2017.