Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading

Synthesis and antibacterial evaluation of 2-substituted-4,5-diphenyl-N-alkyl imidazole derivatives

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ARTICLE INFO

Article history:
Received 10 May 2010
Received in revised form 27 May 2010
Accepted 7 June 2010
Available online 20 June 2010

Keywords:
Antibacterial activity
Imidazole derivatives
N- alkyl imidazole

ABSTRACT

Objective: To synthesis 2-substituted-4,5-diphenyl-N- alkyl imidazole derivatives. and evaluate their antibacterial activity. **Methods:** A mixture of benzil (10 mmol) and ammonium acetate (0.1 mol) (immediately fused) in glacial acetic acid (25 mL) was stirred at 80-100 ℃ for 1 h under nitrogen atmosphere (to prevent incorporation of any atmospheric impurities and moisture). Substituted aldehydes (10 mmol) in glacial acetic acid (5 mL) was added drop-wise over a period of 15-20 min at the same temperature and stirred for another 4 h, the progress of the reaction was monitored by TLC test using ethyl acetate as eluent. The newly synthesized compounds were characterized by IR, ^{1}HNMR, ^{13}CNMR and by mass spectroscopy. **Results:** All the synthesized compounds were confirmed by spectroscopical techniques and evaluated for antimicrobial activity against *Staphylococcus aureus(S. aurius)*, *Bacilus subtilus (B. subtilus)*, and *Escheria coli (E. coli)*. These compounds showed antibacterial activity (zone of inhibition) against *S. aurius* ranged from 3 mm to 9 mmin diameter, *B. subtilus*, 4 -8 mm, and *E. coli* 5 -12 mm. Out of 2a-2e, only 2a and 2b showed some sort of activity but none of them had considerable activity compared with that of the standard. **Conclusions:** All the synthesized compounds show moderate activity against the tested bacteria *S. aurius*, *B. subtilus*, and *E. coli*. So, further structural modification is necessary to improve the antibacterial action of 2-substituted-4,5-diphenyl-N-alkyl imidazole derivatives.

1. Introduction

N-substituted imidazoles represents a class of heterocyclic analogues having valuable pharma-cological properties such as antiparasitic[1], antifungal[2], antimicrobial[3-7], and antidepressant[8] activity. It has been reported that N-alkylimidazoles with the most simple structure posses inhibitory effects on microsomal oxidation[9] cytotoxic[10] and antifungal[3] activity. Some imidazole drugs have surface activity and are able to damage membranes directly when used at a high concentration for a very short time, independently of the culture medium and growth rate. When in direct contact[11,12] imidazoles interact directly with the double lipid layer[13] of the membrane structure, probably by binding to the unsaturated fatty acid part of the phospholipids components of the membrane. Some microorganisms show resistance to imidazole action due to outer membrane modifications[14-16].

The development of new and different antimicrobial agents has been a very important step. The resistance of common pathogens to standard antibiotic therapy is rapidly becoming a major health problem throughout the world. There is continuously increased incidence of multidrug-resistant gram-positive bacteria and infections caused by *staphylococcus aureus (S. aureus)* (multidrug-resistant *S. aureus* and coagulase negative *staphylococcus*), *enterococci*, and *pneumococci* are particularly problematic[14]. Much of the research programmer efforts are oriented toward the design of new and available antimicrobial drugs, but due to unsatisfactory status of present drugs' side effects, and the acquisition of resistance by the infecting organisms to the present drugs[9]. There is a real perceived need for the discovery of new compounds endowed with antibacterial property, possibly acting through mechanisms of action that are distinct from those of the well-known classes of antibacterial agents to which many clinically relevant pathogens are now resistant[10].

Khabnadideh *et al* reported synthesis of N- alkylated derivatives of imidazole as antibacterial agents, antibacterial effects of these compounds were investigated to achieve the relationship between the length of the alkyl chain and antibacterial activity[17]. 2-substituted-

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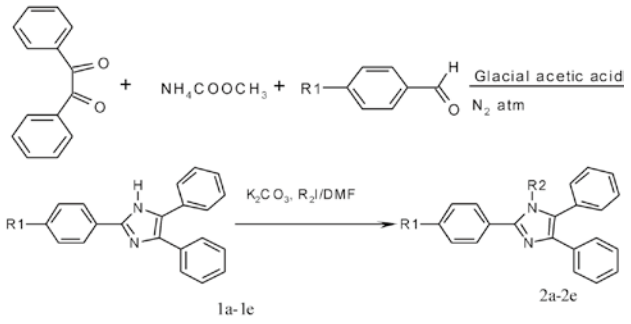
4,5-diphenyl-N-alkyl imidazole derivatives showed antinociceptive and antiinflammatory activities. In this study, antibacterial effects of synthesized compounds were investigated to achieve the effect of the phenyl group and length of the alkyl chain on antibacterial activity. We have now synthesized some new 4,5- disubstituted-N- alkyl imidazole derivatives (2a-2e) incorporating substituted phenyl moiety at position 2nd. These compounds were characterized by their elemental and spectral analyses (IR, ^{1}HNMR, ^{13}CNMRand mass spectra).

2. Material and methods

All chemicals were of analytical grade and were used directly. All melting points were determined in PMP-DM scientific melting point apparatus and were uncorrected. Infrared spectra were recorded on a Perkin Elmer RX-1 model spectrophotometer using KBr pellets. ^{1}HNMR spectra were acquired on a Bruker Avance-2 model spectrophotometer using DMSO as a solvent and TMS as internal reference (chemical shifts in δ, ppm). Mass spectra (MS) were recorded on Schimadzu GC-MS. All products were purified by recrystallisation. The reactions were followed up and the purity of products was carried out on pre-coated TLC plates (Silica gel 60, Merck), visualizing the spots under ultraviolet light. Column chromatography was performed on Merck silicagel (60-120 mesh).

2.1. General procedure for the synthesis of 2(4-substituted-phenyl)-4,5-diphenyl-1H-imidazoles (1a-1e)

A mixture of benzil (10 mmol) and ammonium acetate (0.1 mol) (immediately fused) in glacial acetic acid (25 mL) was stirred at 80- 100 ℃ for 1 h under nitrogen atmosphere (To prevent incorporation of any atmospheric impurities and moisture) . Substituted aldehydes (10 mmol) in glacial acetic acid (5 mL) was added drop-wise over a period of 15-20 min at the same temperature and stirred for another 4 h, the progress of the reaction was monitored by TLC test using ethyl acetate as eluent. After completion of reaction, mixture was allowed to stand at room temperature. The resulting homogenous solution was poured over crushed ice (200 g), The yellow precipitate was collected by filtration and washed with cold water and dried under vacuum.Product was recrystallized from ethyl acetate thrice to afford the pure compound .



R1 = 2a = p- NO_2, 2b = m- NO_2, 2c = p- Cl, 2d = o- Cl, 2e = m- Br, R2 = Butyl

Figure 1. Various steps involved in synthesis of 2-substituted-4,5-diphenyl-N-alkyl imidazole derivatives.

Compound (1a): m.p. 144-148 ℃ yield 70% ^{1}H NMR DMSO: 7.22-7.74 (m, 12H, Ar-H), 8.12 (d, 2H, Ar-H), 12.9 (s, 1H, N-H), IR (cm^{-1}) 3068.0, 3014.8, 1674, 1348, 758. Compound (1b): m.p. 135-140 ℃ yield 62% ^{1}H NMR DMSO: 7.22-7.58 (m, 11H, Ar-H), 8.11 (d, 1H, Ar-H), 8.55 (s, 1H, Ar-H), 13.1 (s, 1H, N-H), IR (cm^{-1}) 3066.0, 3018, 1680, 1337, 750. Compound (1c): m.p. 118-122 ℃ yield 55% ^{1}H NMR DMSO: 7.3-7.41 (m, 12H, Ar-H), 7.51-7.59 (m, 2H, Ar-H), 13.1 (s, 1H, N-H), IR (cm^{-1}) 3054.8, 1594.6, 1092.9, 771.9. Compound (1d): m.p. 124-129 ℃yield 53% ^{1}H NMR DMSO: 7.20-7.52 (m, 14H, Ar-H), 12.9 (s, 1H, N-H), IR (cm^{-1}) 3045.1, 1590.2, 1090.2, 768.7. Compound (1e): m.p. 108-112 ℃ yield 53%^{1}H NMR DMSO: 7.22-7.34 (m, 13H, Ar-H), 7.67 (s, 1H, Ar-H), 13.1 (s, 1H, N-H), IR (cm^{-1}) 3051.0, 1595.2, 1071.5, 892.0, 822.9, 747.1.

2.2. General procedure for the synthesis of 1-butyl- 2 (4-substituted phenyl) -4,5-diphenyl -1H-imidazoles (2a-2e)

Compounds 2a-2e are synthesized from 1a-1e, K_2CO_3 (7 mmol) was added to intermediates (1a-1e) (5.8 mmol), in DMF (25 mL) at 0 ℃ under nitrogen. After 15 min, butyl iodide (6 mmol) was added in drop wise at 0 ℃ and continued stirring for 2 h, and then the mixture was stirred overnight at room temperature and poured over crushed ice (150 g). Reaction mixture was filtered and washed with ice cold water several times to remove DMF and dried in an oven. Product was recrystallized from ethyl acetate thrice to afford the pure compound. Product was purified by column chromatography using ethyl acetate/n-hexane as eluent.

Compound (2a). ^{1}H NMR DMSO: 0.64 (t, 3H, -CH_3), 0.86-0.94 (m, 2H, -CH_2), 1-1.3 (m, 2H, -CH_2), 3.72-3.84 (t, 2H, -CH_2), 7.18-7.41 (m, 10H, Ar-H), 7.61 (d, 2H, Ar-H), 8.1 (d, 2H, Ar-H); IR (cm^{-1}) 3068.0, 2995.0, 2970.0, 1674, 1348.29, 758.05; ^{13}C NMR (50 MHz, $CDCl_3$) 11.2 (-CH_3), 17.8 (-CH_2), 28.1 (-CH_2), 34.2 (-CH_2), 118.7, 122.2, 128.2, 128.3, 133.2, 133.4, 142.1, 152.3 (Aryl-C), 128.2, 130.1, 148.2 (imidazole-C); MS: (M+, 100) 396; Anal. Calcd. for $C_{25}H_{23}N_3O_2$: C 75.55, H 5.83, N 10.57 Found: C 75.48, H 5.75, N 10.51.

Compound (2b). ^{1}H NMR DMSO: 0.77 (t, 3H, -CH3), 1.12 (m, 2H, -CH_2), 1.44 (m, 2H, -CH_2), 2.99 (t, 2H, -CH_2),7.32-7.67 (m, 11H, Ar-H), 8.33 (d, 1H, Ar-H), 8.50 (s, 1H, Ar-H); IR (cm^{-1}) 3057.0, 2965.3, 1680, 1522.2, 1347.8, 970.5, 805.5, 766.1; ^{13}C NMR (50 MHz, $CDCl_3$) 11.8 (-CH_3), 16.8 (-CH_2), 30.1 (-CH_2), 31.2 (-CH_2), 119.2, 119.9, 126.2, 129.2, 133.1, 133.3, 145.3, 155.3 (Aryl -C), 126.2, 133.1, 153.2 (imidazole-C), MS: (M+, 100) 396; Anal. Calcd. for $C_{25}H_{23}N_3O_2$: C 75.55, H 5.83, N 10.57 Found C 75.42, H 5.79, N 10.53.

Compound (2c). ^{1}H NMR DMSO: 0.63 (t, -CH_3), 1.12 (m, 2H, -CH_2), 1.32 (m, 2H, -CH_2), 3.82 (t, 2H, -CH_2), 7.33-7.48 (m, 12H, Ar-H), 7.64-7.74 (m, 2H, Ar-H); IR (cm^{-1}) 3058.8, 2955.8, 1590.8, 1089.9, 768.1; ^{13}C NMR (50 MHz, $CDCl_3$) 11.2 (-CH_3), 15.8 (-CH_2), 30.3 (-CH_2), 34.2 (-CH_2), 117.1, 118.9, 128.3, 128.7, 132.6, 132.3, 133.3, 135.3 (Aryl-C), 125.3, 132.8, 150.1 (imidazole-C), MS: (M+, 100) 386; Anal. Calcd. for $C_{25}H_{23}N_2Cl$: C 77.61, H 5.99, N 7.24 Found: C 77.57, H 5.84, N 7.19.

Compound (2d). ^{1}H NMR DMSO: 0.68 (t, -CH_3), 1.02 (m, 2H, -CH_2), 1.28 (m, 2H, -CH_2), 3.19 (t, 2H, -CH_2),7.20-7.52 (m, 14H, Ar-H), 3045.1, 1590.2, 1090.2, 768.7; ^{13}C NMR (50 MHz, $CDCl_3$) 10.8 (-CH_3), 13.3 (-CH_2), 28.2 (-CH_2), 32.4 (-CH_2), 118.2, 119.8, 119.3, 125.2, 130.2, 131.1, 137.7, 139.3 (Aryl-C), 122.2, 134.2, 154.8 (imidazole-C), MS: (M+, 100) 386; Anal. Calcd. for $C_{25}H_{23}N_2Cl$: C 77.61, H 5.99, N 7.24 Found: C 77.58, H 5.87, N 7.16.

Compound (2e). ^{1}H NMR DMSO: 7.18–7.26 (m, 4, Ar–H), 7.34–7.42 (m, 10H, Ar–H), 7.71 (s, 1H, Ar–H); IR (cm^{-1}) 3053.2, 2953.8, 1615.2, 1071.8, 890.0, 742.2; ^{13}C NMR (50 MHz, $CDCl_3$) 10.2 ($-CH_3$), 12.9 ($-CH_2$), 22.4 ($-CH_2$), 29.4 ($-CH_2$), 118.2, 120.8, 124.3, 124.2, 131.2, 131.1,132.3,132.8, 135.7, 138.3, (Aryl–C), 126.2, 133.2, 152.9 (imidazole–C), MS: (M+, 100) 430; Anal. Calcd. for $C_{25}H_{23}N_2Br$: C 69.61, H 5.37, N 6.49 Found: C 69.56, H 5.32, N 6.38.

2.3. Antibacterial assays

The *in vitro* antibacterial activity[18] of synthesized compounds 4a–4e was carried out for 24 h culture of five selected bacteria. The bacteria organisms used were *Escheria coli (E. coli)* , *S. aureus*, *Bacilus subtilus (B. subtilus)*. All the test organisms are clinical isolates of human pathogens obtained from the IMTECH Chandigarh, India. Cultures were brought to laboratory conditions by resuscitating the organism in buffered peptone broth and thereafter agar medium and incubated at 37 ℃ for 24 h. The antibacterial activity was performed by cylinder wells diffusion technique. The medium (7 g nutrient agar in 250 mL distilled water, auto claved at 115 ℃ for 15 min) was cooled to 50 ℃. 20 mL of the medium was poured into a sterile Petri dish and allowed to solidify. It was allowed to stay for 8 h and observed for contamination.

The sterility of the medium was tested after sterilization 10 mg of synthesized compounds was dissolved in 10 mL of DMF and made and further diluted to give a concentration of 50–150 μg/mL, a colony of each test organism was subcultured on nutrient broth and incubated at 37 ℃ for 8 h. This was then used to flood the agar plates. After setup the culture media, a sterilized glass tubes (5 mm diameter) was used aseptically to scoop out the media to make wells, two drops (0.1 mL) of the sample solution were filled into these wells aseptically. The plates were incubated at 37 ℃ for 24 h. After incubation, the Zone of inhibition was then measured and compared with that of the standard (norfloxacin).

3. Results

All the synthesized compounds were confirmed by spectroscopical techniques and evaluated for antimicrobial activity against *S. aurius*, *B. subtilus* and *E. coli*. The substituents of the compounds with physicochemical properties are given in Table 1. These compounds showed antibacterial activity (zone of inhibition) against *S. aurius* ranged from 3–9 mm in diameter, *B. subtilus*, 4 –8 mm, and *E. coli* 5 –12. Out of 2a–2e, only 2a and 2b showed some sort of activity but none of them had considerable activity than the standard (Table 2).

Table 1
Physicochemical properties of compounds 2a–2e.

Compounds	R1	R2	Molecular formula	Melting point (℃)	Color
2a	*p*– NO_2	$-CH_2-CH_2-CH_2-CH_3$	$C_{25}H_{23}N_3O_2$	168–174	Dark yellow
2b	*m*– NO_2	$-CH_2-CH_2-CH_2-CH_3$	$C_{25}H_{23}N_3O_2$	166–174	Light yellow
2c	*p*– Cl	$-CH_2-CH_2-CH_2-CH_3$	$C_{25}H_{23}N_2Cl$	145–148	Off white colour
2d	*o*– Cl	$-CH_2-CH_2-CH_2-CH_3$	$C_{25}H_{23}N_2Cl$	167–170	White
2e	*m*– Br	$-CH_2-CH_2-CH_2-CH_3$	$C_{25}H_{23}N_2Br$	119–125	Yellowish white

Table 2
Antibacterial activity of 1–butyl– 2 (4–substituted phenyl) –4,5–diphenyl –1H–imidazoles derivatives-zone of inhibition (mm, %).

Compounds	Zone of inhibition					
	S. aurius		*B. subtilis*		*E. coli*	
	50 (μg/mL)	150 (μg/mL)	50 (μg/mL)	150 (μg/mL)	50 (μg/mL)	150 (μg/mL)
2a	5 (23.09)	9 (42.85)	4 (19.04)	8 (38.09)	7 (33.33)	9 (42.85)
2b	3 (14.28)	7 (33.33)	4 (19.04)	7 (33.33)	6 (28.57)	9 (42.85)
2c	5 (23.09)	6 (28.57)	6 (28.57)	7 (33.33)	5 (23.09)	8 (38.09)
2d	5 (23.09)	6 (28.57)	6 (28.57)	6 (28.57)	5 (23.09)	8 (38.09)
2e	4 (19.04)	7 (33.33)	4 (19.04)	7 (33.33)	5 (23.09)	8 (38.09)
A*	21	–	21	–	21	–

A* = Norfloxacin at concentration 50 μg/mL.

4. Discussion

In this work, the intermediate 2 (4–substituted–phenyl)–4,5–diphenyl– 1H–imidazoles (1a–1e) was synthesized using the reported procedure[19]. Wen–Long Pan reaction was carried out by reacting benzil and ammonium acetate with substituted aldehydes in glacial acetic acid (1a–

1e). These 2(4-substituted-phenyl)-4,5-diphenyl-1H imidazoles derivatives react with butyl iodide give the corresponding 1-butyl- 2 (4-substituted phenyl) -4,5-diphenyl -1H-imidazoles derivatives (2a-2e). The best yield of the compounds is achieved when the reaction is carried out at 0 ℃ in presence of DMF. These reactions are summarized in Figure 1. Yields were moderate to fair (50-77%). The purity of the compounds was monitored by TLC and the synthesized compounds were recrystallized by appropriate solvents (Ethyl acetate). All final substituted imidazole analogues are analyzed by different spectroscopic technique such as IR, NMR, mass spectroscopy and by elemental analysis. These derivatives were further evaluated for antibacterial activity. Literature survey revealed that 4,5 disubstituted N- alkylated imidazole ring has anti-inflammatory and analgesic activities. Antibacterial activity of synthesized compounds was much inferior compare to standard drug norfolxacin.

Most of the newly synthesized compounds were tested for their antibacterial activity in vitro against bacterial strains such as *E. coli*, *B. subtillis* and *S. aurius*, employing the nutrient agar disc diffusion method 14 at 50 and 150 μg/mL concentrations. Norfloxacin shows inhibition zone of 21 mm but only 2a had maximum 12 mm zone of inhibition against *E. coli* the activity of 2a and 2b is due to presence of chain length of 4 C atom and NO_2 group at phenyl ring. The rest of the compounds were found to be moderately active, slightly active or inactive against the tested microorganisms. The results of antimicrobial testing are compared with those of standards norfloxacin as antibacterial agents. For each biological activity test, three to four experiments were performed and the average zone of inhibition is reported in this work.

All the synthesized compounds are shown moderate activity against the tested bacteria *S. aurius*, *B. subtilus*, and *E. coli*. So, further structural modification is necessary to improve the antibacterial action of 2-substituted-4,5-diphenyl-N-alkyl imidazole derivatives

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

One of the authors Abhishek K jain is thankful to AICTE, New Delhi for providing Research Fellowship.

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