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Synthesis of Mono, Bis-2-(2-Arylideneaminophenyl) Indole Azomethines as Potential Antimicrobial Agents

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A series of mono and bis 2-2-(arylidineaminophenyl)indole azomethines have been synthesized by a condensation reaction of 2-(2-amino phenyl) indole with various mono and diketones R-CO-R[1] /R-CO-X-CO-R[1] (1:1/2:1 ratio) in ethanol media. The synthesized azomethines were characterized via IR, ^{1}H-NMR, ^{13}C-NMR, MS and elemental analysis. The antimicrobial activity of these compounds against different bacteria and fungi was also evaluated.

Key words: Azomethines, Synthesis, Antibacterial activity, Antifungal activity

INTRODUCTION

Heterocycles play a central role in most bioactive molecules and in their manifestation of bioactivity. Despite the ever-increasing number of new bioactive compounds, nitrogen heterocycles and their derivatives have now developed into a major class which is fulfilling a seminal role in synthetic organic and medicinal chemistry (Kaplancikli et al., 2004; Jung et al., 2006; Habib et al., 2007; Mostafa et al., 2008). Additionally, azomethines derived from various heterocycles have been well-recognized for their pharmacological properties including anticonvulsant (Popp et al., 1980; Küçükgüzel et al., 2004), analgesic (Sarangapani and Reddy, 1996), cytotoxic (Tarafder et al., 2002), antiproliferative (Vicini et al., 2003), antidepressant (Singh et al., 1997), anti-inflammatory (Andreani and Maselli, 1977) and antimicrobial (Pignatello et al., 1994) activities. Consequently, development of new beneficial azomethines is now attracting the attention of the medicinal chemist. Literature surveys reveal that combination of two or more moieties into one is a common procedure for manipulation in medicinal chemistry and this can possibly result in augmenting the activity towards infectious microorganisms. Moreover, the importance of the indole nucleus is well established in the field of synthetic as well as medicinal chemistry. Indole derivatives offer a high degree of structural diversity and have proven to be broadly useful as therapeutic agents. Usually, indole and its analogs constitute the active class of compounds possessing a wide spectrum of biological activities, such as antiinflammatory (Zheng et al., 2007), antimicrobial (Gurkok et al., 2009), antibacterial (Sharma et al., 2006), anticonvulsant (Gitto et al., 2009) and cardiovascular (Yorikane et al., 1991). In fact, a number of drugs such as indolmycin (Hurdle et al., 2004), reserpine (Krönig et al., 1997), vincristine (Mihelic et al., 2007), the 5-HT3 receptor antagonist ondansetron (Clavel et al., 1995) and 5HT1 receptor agonist sumatriptan (Sakai et al., 2008) have an indole nucleus in their structures. In recent years, design and synthesis of pharmacologically relevant heterocyclic molecules having indole subunits have proven to be a promising strategy in the search for new pharmaceutical lead structures. In view of the above and in continuation of our earlier work on the synthesis of new beneficial azomethines (Reddy et al., 2009; Rohini et al., 2009; Shanker et al., 2009a), finding indole-induced azomethines was under taken in the hope of getting better

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bioactive agents. Here, we present a convenient synthesis approach and antimicrobial screening of novel indole azomethines.

MATERIALS AND METHODS

All solvents used were of analytical grade. Melting points were determined in open capillary tubes and are uncorrected. TLC analysis was done using precoated silica gel plates and visualization was done using iodine vapors. Micro analytical (C, N, H) data was obtained by using a Perkin-Elmer 2400 CHN elemental analyzer. The IR spectra were recorded in KBr pellets on a Perkin-Elmer-283 spectrophotometer (ν in cm^{-1}). ^{1}H-NMR spectra (at 400 MHz) and ^{13}C-NMR (at 100 MHz) spectra were acquired on a Bruker NMR spectrometer. FAB mass spectra were recorded on a Finnigan-MAT 1020 instrument. MALDI Autoflex time-of-flight mass spectrometer (Bruker Daltonic) was used for MS analyses. *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus pyogenes* (Gram positive), *Salmonella typhimurium, Escherichia coli* and *Klebsiella pneumonia* (Gram negative) bacteria as well as *Aspergillus niger, Candida albicans, Trichoderma viridae* fungi were used in the antimicrobial assays.

General procedure for the preparation of 2-(o-aminophenyl)indole (A)

The starting material, 2-(o-aminophenyl)indole (**A**, lit. m.p. 146°C) was obtained by Fischer indole cyclization of phenylhydrazene and 2-aminoacetophenone using a mixture of methane sulfonic acid and phosphorus pentoxide at 85°C. Reactions were carried out according to a reported procedure (Billimoria and Cava, 1994). Methanesulfonic acid (100 mL) by heating to 80°C, then phosphorus pentoxide (13.5 g) was added to it with stirring until it dissolved completely. The phenylhydrazone of 2-aminoacetophenone (10.0 g, 44.4 mmol) (Kiang et al., 1956) was slowly added maintaining the temperature between 80 and 100°C. The solution was then further heated at 85°C for 30 min. The reaction mixture was cooled to room temperature and poured over crushed ice containing sodium hydroxide (65.0 g). The solid precipitate was filtered, washed with water and dried to afford the crude product which was crystallized from ethanol.

Procedure for the preparation of mono-2-(2-arylideneaminophenyl) indoles (Az1-6)

To a solution of 2-(o-aminophenyl)indole (**A**, 0.416 g, 2 mmol) in 20-40 mL of ethanol were added the appropriate ketone (2 mmol of $RCOR^1$ in ethanol) and a few drops of acetic acid with vigorous stirring. The resulting solution was refluxed for 3 h. After cooling, the deposited solid product was collected by filtration and recrystalized with an ethanol/dichloromethane-methanol mixture.

Procedure for the preparation of bis-2-(2-arylideneaminophenyl)indoles (Az7-13)

0.832 g, 4 mmol of 2-(o-aminophenyl)indole (**A**) was dissolved in 20-40 mL of ethanol. To this solution, 30 mL of ethanolic $RCO\text{-}X\text{-}COR^1$ solution (2 mmol) and a few drops of acetic acid were added. The reaction mixture was refluxed for 2 h after which the compound was obtained. The product was collected and recrystalized as indicated above.

[2-(1H-Indol-2-yl)-phenyl]-(1-phenyl-ethylidene)-amine (Az1)

Yield 75%, m.p. 211-213°C; ^{1}H-NMR ($CDCl_3$): δ 2.32 (3H, S, -CH_3), 6.82 (1H, t, Ar, J = 7.4 Hz), 7.08-7.16 (4H, m, Ar), 7.29-7.42 (7H, m, Ar), 7.61 (1H, d, Ar, J = 7.3 Hz), 7.94 (1H, d, Ar, J = 7.3 Hz), 8.85 (1H, s, indolyl-NH); ^{13}C-NMR ($CDCl_3$): δ 19.2 (1C, -CH_3), 114.0, 118.1, 120.5, 123.2, 125.6, 130.5, 131.3, 132.2, 136.5, 141.7, 142.6, 143.8, 146.3, 165.8 (21C, Ar-C). IR (KBr) cm^{-1}: 3290, 2868, 1630, 1590, 1580, 1128. MS m/z: 310. Anal. Calcd for $C_{22}H_{18}N_2$: C, 85.13; H, 5.85; N, 9.03%. Found: C, 85.18; H, 5.87; N, 9.05%.

2-{1-[2-(1H-Indol-2-yl)-phenylimino]-ethyl}-phenol (Az2)

Yield 79%, m.p. 205-207°C; ^{1}H-NMR ($CDCl_3$): δ 2.34 (3H, S, -CH_3), 6.85-7.20 (6H, m, Ar), 7.26-7.37 (5H, m, Ar), 7.61 (1H, d, Ar, J = 7.3 Hz), 7.95 (1H, d, Ar, J = 7.3 Hz), 8.92 (1H, s, indolyl-NH), 10.21 (1H, s, OH); ^{13}C-NMR ($CDCl_3$): δ 19.4 (1C, -CH_3), 114.7, 117.5, 118.4, 123.8, 126.2, 130.6, 132.2, 133.6, 136.2, 141.2, 142.6, 143.5, 145.4, 153.7, 167.8 (21C, Ar-C). IR (KBr) cm^{-1}: 3420, 3295, 2860, 1625, 1590, 1578, 1130. MS m/z: 326. Anal. Calcd for $C_{22}H_{18}N_2O$: C, 80.96; H, 5.56; N, 8.58%. Found: C, 80.95; H, 5.61; N, 8.55%.

[2-(1H-Indol-2-yl)-phenyl]-(1-naphthalen-2-yl-ethylidene)-amine (Az3)

Yield 80%, m.p. 215-217°C; ^{1}H-NMR ($CDCl_3$): δ 2.31 (3H, S, -CH_3), 6.83 (1H, t, Ar, J = 7.4 Hz), 7.08-7.18 (6H, m, Ar), 7.21-7.65 (8H, m, Ar), 7.95 (1H, d, Ar, J = 7.3 Hz), 8.90 (1H, s, indolyl-NH); ^{13}C-NMR ($CDCl_3$): δ 19.5 (1C, -CH_3), 114.4, 118.2, 123.1, 123.8, 126.2, 130.5, 130.8, 131.2, 132.5, 136.8, 141.6, 142,5, 143.8, 146.3, 166.3 (25C, Ar-C). IR (KBr) cm^{-1}: 3260, 2860, 1635, 1590, 1575, 1126. MS m/z: 360. Anal. Calcd for $C_{26}H_{20}N_2$: C, 86.64; H, 5.59; N, 7.77%. Found: C, 86.66; H, 5.61; N, 7.81%.

Benzhydrylidene-[2-(1H-indol-2-yl)-phenyl]-amine (Az4)
Yield 76%, m.p. 199-201°C; ^{1}H-NMR ($CDCl_3$): δ 2.31 (3H, S,-CH_3), 6.82 (1H, t, Ar, J = 7.4 Hz), 7.06-7.34 (9H, m, Ar), 7.57-7.65 (5H, m, Ar), 7.94 (1H, d, Ar, J = 7.3 Hz), 8.87 (1H, s, indolyl-NH); ^{13}C-NMR ($CDCl_3$): δ 19.2 (1C, -CH_3), 113.4, 117.6, 123.0, 124.3, 126.5, 130.5, 131.8, 132.2, 136.9, 141.0, 142.2, 145.0, 150.8, 165.9 (26C, Ar-C). IR (KBr) cm^{-1}: 3300, 2875, 1630, 1595, 1575, 1100. MS *m/z*: 372. Anal. Calcd for $C_{27}H_{20}N_2$: C, 87.07; H, 5.41; N, 7.52%. Found: C, 87.10; H, 5.45; N, 7.55%.

[2-(1H-Indol-2-yl)-phenyl]-(1-pyridin-2-yl-ethylidene)-amine (Az5)
Yield 78%, m.p. 221-223°C; ^{1}H-NMR ($CDCl_3$): δ 2.34 (3H, S, -CH_3), 6.85-7.12 (3H, m, Ar), 7.16-7.33 (5H, m, Ar), 7.61-7.86 (3H, m, Ar), 7.95 (1H, d, Ar, J = 7.3 Hz), 8.71 (1H, d, J = 5.25 Hz), 8.90 (1H, s, indolyl-NH); ^{13}C-NMR ($CDCl_3$): δ 19.6 (1C, -CH_3), 113.3, 114.2, 119.4, 123.6, 125.1, 131.6, 137.2, 140.7, 142.4, 143.6, 147.0, 160.3, 167.1 (20C, Ar-C). IR (KBr) cm^{-1}: 3290, 2870, 1635, 1615, 1585, 1125. MS *m/z*: 311. Anal. Calcd for $C_{21}H_{17}N_3$: C, 81.00; H, 5.50; N, 13.49%. Found: C, 81.05; H, 5.55; N, 13.55%.

[2-(1H-Indol-2-yl)-phenyl]-[1-(6-methyl-pyridin-2-yl)-ethylidene]-amine (Az6)
Yield 75%, m.p. 228-230°C; ^{1}H-NMR ($CDCl_3$): δ 2.29 (3H, S, -CH_3), 2.34 (3H, S, -CH_3), 6.70-7.21 (5H, m, Ar), 7.29-7.32 (3H, m, Ar), 7.62 (1H, d, Ar, J = 7.3 Hz), 7.76-7.81 (2H, m, Ar), 7.93 (1H, d, Ar, J = 7.3 Hz), 8.89 (1H, s, indolyl-NH); ^{13}C-NMR ($CDCl_3$): δ 20.2 (2C, -CH_3), 113.5, 114.2, 118.2, 123.4, 126.2, 130.7, 130.8, 131.7, 132.7, 136.5, 140.1, 142,8, 143.6, 146.7, 149.4, 161.2, 166.4 (20C, Ar-C). IR (KBr) cm^{-1}: 3278, 2862, 1635, 1618, 1580, 1120. MS *m/z*: 325. Anal. Calcd for $C_{22}H_{19}N_3$: C, 81.20; H, 5.89; N, 12.91%. Found: C, 81.42; H, 5.87; N, 12.95%.

N1-[1-(2-[2-(1H-2-indolyl)phenyl]ethanimidoyl-phenyl)ethylidene]-2-(1H-2-indolyl)aniline (Az7)
Yield 80%, m.p. 243-245°C; ^{1}H-NMR ($CDCl_3$): δ 2.25 (6H, S, -CH_3), 6.81-7.20 (10H, m, Ar), 7.26-7.45 (10H, m, Ar), 7.94 (2H, d, Ar, J = 7.3 Hz), 8.92 (2H, s, -NH); ^{13}C-NMR ($CDCl_3$): δ 20.5 (2C, -CH_3), 113.1, 114.6, 119.7, 123.2, 124.7, 125.2, 127.2, 128.3, 130.5, 131.2, 133.9, 136.3, 141.5, 142.2, 144.8, 146.5, 165.8, 169.5 (36C, Ar-C). IR (KBr) cm^{-1}: 3300, 2860, 1628, 1605, 1582, 1325, 1115. MS *m/z*: 542. Anal. Calcd for $C_{38}H_{30}N_4$: C, 84.10; H, 5.57; N, 10.32%. Found: C, 84.16; H, 5.62; N, 10.37%.

N1-[1-(3-[2-(1H-2-indolyl)phenyl]ethanimidoyl-phenyl)ethylidene]-2-(1H-2-indolyl)aniline (Az8)
Yield 81%, m.p. 205-207°C; ^{1}H-NMR ($CDCl_3$): δ 2.24 (6H, S, -CH_3), 6.81-6.87 (4H, m, Ar), 7.08-7.41 (12H, m, Ar), 7.61-7.96 (6H, m, Ar), 8.92 (2H, s, -NH); ^{13}C-NMR ($CDCl_3$): δ 21.2 (2C, -CH_3), 113.5, 114.1, 122.6, 124.2, 126.7, 128.2, 130.9, 132.1, 133.9, 136.1, 140.4, 142.8, 145.5, 146.1, 165.4, 168.5 (36C, Ar-C). IR (KBr) cm^{-1}: 3290, 2878, 1648, 1620, 1580, 1324. MS *m/z*: 542. Anal. Calcd for $C_{38}H_{30}N_4$: C, 84.10; H, 5.57; N, 10.32%. Found: C, 84.11; H, 5.62; N, 10.35%.

N1-[1-(4-[2-(1H-2-indolyl)phenyl]ethanimidoyl-phenyl)ethylidene]-2-(1H-2-indolyl)aniline (Az9)
Yield 79%, m.p. 236-238°C; ^{1}H-NMR ($CDCl_3$): δ 2.23 (6H, S, -CH_3), 6.81-6.84 (4H, m, Ar), 7.06-7.15 (6H, m, Ar), 7.21-7.45 (10H, m, Ar), 7.95 (2H, d, Ar, J = 7.3 Hz), 8.86 (2H, s, -NH); ^{13}C-NMR ($CDCl_3$): δ 20.4 (2C, -CH_3), 113.2, 114.5, 123.5, 125.6, 127.3, 128.3, 130.5, 131.2, 132.2, 137.4, 142,6, 144.2, 162.3, 168.6 (36C, Ar-C). IR (KBr) cm^{-1}: 3300, 2865, 1625, 1608, 1590, 1128. MS *m/z*: 542. Anal. Calcd for $C_{38}H_{30}N_4$: C, 84.10; H, 5.57; N, 10.32%. Found: C, 84.15; H, 5.55; N, 10.33%.

N1-[2-[2-(1H-2-indolyl)phenyl]imino-1,2-diphenyl-ethylidene]-2-(1H-2-indolyl) aniline (Az10)
Yield 82%, m.p. 195-197°C; ^{1}H-NMR ($CDCl_3$): δ 6.81-7.14 (6H, m, Ar), 7.20-7.31 (12H, m, Ar), 7.42-7.60 (8H, m, Ar), 7.92 (2H, d, Ar, J = 7.3 Hz), 8.91 (2H, s, indolyl-NH); ^{13}C-NMR ($CDCl_3$): δ 114.6, 118.2, 123.3, 123.4, 126.2, 130.4, 130.6, 131.2, 132.8, 136.6, 140.7, 142,6, 143.9, 162.4, 165.6 (42C, Ar-C). IR (KBr) cm^{-1}: 3295, 2875, 1620, 1606, 1578, 1125. MS *m/z*: 590. Anal. Calcd for $C_{42}H_{30}N_4$: C, 85.40; H, 5.12; N, 9.48%. Found: C, 85.42; H, 5.15; N, 9.50%.

N1-[3-[2-(1H-2-indolyl)phenyl]imino-1,3-diphenyl-propylidene]-2-(1H-2-indolyl) aniline (Az11)
Yield 80%, m.p. 213-215°C; ^{1}H-NMR ($CDCl_3$): δ 2.34 (2H, S, -CH_2-), 6.82 (2H, t, Ar, J = 7.4 Hz), 7.10-7.16 (4H, m, Ar), 7.26-7.33 (12H, m, Ar), 7.38-7.80 (8H, m, Ar), 7.94 (2H, d, Ar, J = 7.3 Hz), 8.92 (2H, s, indzolyl-NH); ^{13}C-NMR ($CDCl_3$): δ 35.5 (1C, -CH_2-), 113.7, 114.3, 118.6, 122.4, 125.2, 130.5, 132.6, 133.3, 136.5, 141.9, 142.2, 143.5 160.3, 165.6 (42C, Ar-C). IR (KBr) cm^{-1}: 3290, 2880, 1620, 1610, 1585, 1128. MS *m/z*: 604. Anal. Calcd for $C_{43}H_{32}N_4$: C, 85.40; H, 5.33; N, 9.26%. Found: C, 85.45; H, 5.33; N, 9.25%.

N1-[4-[2-(1H-2-indolyl)phenyl]imino-1,4-diphenyl-butylidene]-2-(1H-2-indolyl)aniline (Az12)
Yield 76%, m.p. 222-224°C; ^{1}H-NMR ($CDCl_3$): δ 2.32 (4H, S, -CH_2-), 6.83 (2H, t, Ar, J = 7.4 Hz), 7.08-7.15

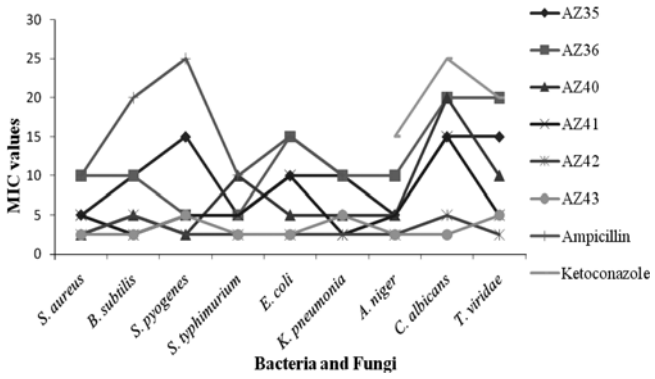


Fig. 1. Comparison of MIC values (in µg/mL) of indoloazomethines and standards against different bacteria and fungi

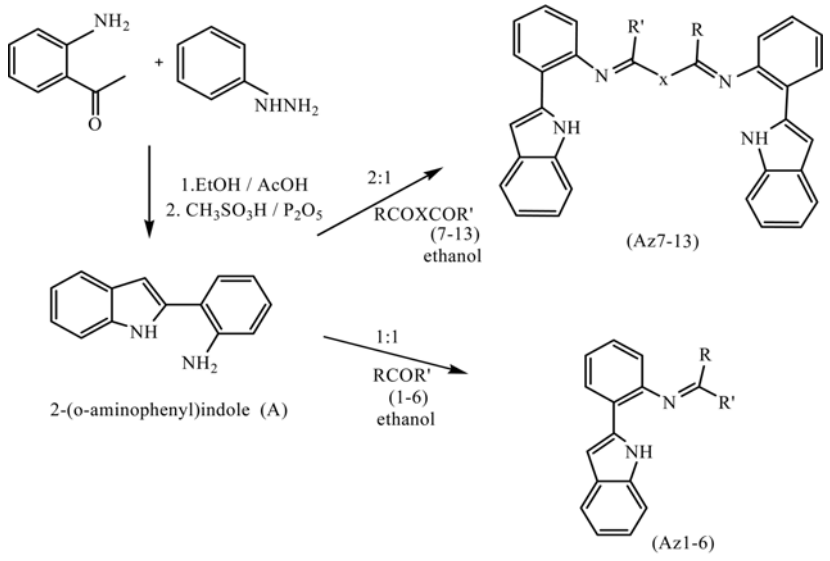
(5H, m, Ar), 7.21-7.36 (9H, m, Ar), 7.32-7.65 (10H, m, Ar), 7.95 (2H, d, Ar, J = 7.3 Hz), 8.92 (2H, s, indolyl-NH); ^{13}C-NMR ($CDCl_3$): δ 31.2 (2C, -CH_2-CH_2-), 113.4, 114.6, 118.2, 123.4, 124.1, 126.6, 130.4, 132.7, 136.1, 141.8, 142.4, 143.2, 156.4, 165.9 (42C, Ar-C). IR (KBr) cm^{-1}: 3285, 2870, 1635, 1615, 1580, 1130. MS m/z: 618. Anal. Calcd for $C_{44}H_{34}N_4$: C, 85.41; H, 5.54; N, 9.05%. Found: C, 85.40; H, 5.56; N, 9.12%.

N1-[1-(6-[2-(1H-2-indolyl)phenyl]ethanimidoyl-2-pyridyl)ethylidene]-2-(1H-2-indolyl)aniline (Az13) Yield 75%, m.p. 212-214°C; ^{1}H-NMR ($CDCl_3$): δ 2.18 (6H, S, -CH_3), 6.82-7.15 (5H, m, Ar), 7.21-7.32 (6H, m, Ar), 7.36-7.41 (6H, m, Ar), 7.85-7.92 (4H, m, Ar), 8.86 (2H, s, -NH); ^{13}C-NMR ($CDCl_3$): δ 20.2 (2C, -CH_3), 113.2, 114.6, 121.3, 123.2, 124.6, 127.1, 128.4, 130.1, 132.6, 140.5, 142.6, 144.2, 146.2, 152.7, 164.1, 168.2 (35C, Ar-C). IR (KBr) cm^{-1}: 3290, 2865, 1630, 1610, 1578, 1126. MS m/z: 543. Anal. Calcd for $C_{37}H_{29}N_5$: C, 81.74; H, 5.38; N, 12.88%. Found: C, 81.75; H, 5.39; N, 12.95%.

RESULTS AND DISCUSSION

The key indole derivative (**A**, 2-o-aminophenylindole) was obtained by the method described by Billimoria and Cava. The target compounds were synthesized by the condensation between 2-(o-aminophenyl)indole and various mono, diketones R-CO-R^1 /R-CO-X-CO-R^1 (in a 1:1/2:1 ratio). The general strategy for the synthesis of mono and bis-2-(2-arylideneaminophenyl) indoles is illustrated in Scheme 1. All newly synthesized compounds were analyzed by different spectroscopic technique such as IR, ^{1}H-NMR, ^{13}C-NMR, mass spectroscopy and by elemental analysis.

The IR spectrum of **A** exhibited an absorption band at 3380-3440 cm^{-1} assigned to -NH_2 group and in the spectra of **A** and azomethine derivatives Az1-13 two absorption bands in the regions 3330-3400 cm^{-1} and 1540-1560 cm^{-1} were assigned to -NH stretching and



RCOR';

1) 1-Phenyl-ethanone
2) 1-(2-Hydroxy-phenyl)-ethanone
3) 1-Naphthalen-2-yl-ethanone
4) Diphenyl-methanone
5) 1-Pyridin-2-yl-ethanone
6) 1-(6-Methyl-pyridin-2-yl)-ethanone

RCO-X-COR';

7) 1-(2-Acetyl-phenyl)-ethanone
8) 1-(3-Acetyl-phenyl)-ethanone
9) 1-(4-Acetyl-phenyl)-ethanone
10) 1,2-Diphenyl-ethane-1,2-dione
11) 1,3-Diphenyl-propane-1,3-dione
12) 1,4-Diphenyl-butane-1,4-dione
13) 1-(6-Acetyl-pyridin-2-yl)-ethanone

Scheme 1. Synthetic route of mono, bis-2-(2-arylideneaminophenyl) indoles

bending vibrations of the indolyl ring, respectively (Talbi et al., 1997). In all azomethines the characteristic IR peaks for the -CHO and -NH_2 groups at 1680-1695 cm^{-1} and 3380-3440 cm^{-1} are absent and the characteristic peak for the CH=N around 1610-1635 cm^{-1} is observed, which indicates effective condensation of the aldehyde group with the amino group of the key indole derivative (2-o-aminophenylindole) (Amudha et al., 1999). The IR spectrum of an azomethine (Az8) is presented in Fig. 2.

The ^{1}H-NMR spectra of all compounds were recorded in $CDCl_3$ using TMS as the internal standard. The ^{1}H-NMR spectra of compound **A** showed signals at δ 6.4 ppm as a singlet for (-NH_2), δ 8.48 ppm as a singlet for the (-NH) group, which confirmed the structure (Billimoria and Cava, 1994). Formation of azomethines from **A** was confirmed by disappearance of signals at δ 6.4 ppm due to NH_2 protons of compound **A** and appearance of signals around δ 7.9-8.1 ppm that corresponded to -CH=N protons. The ^{1}H-NMR spectrum of an azomethine (Az8) is presented in Fig. 3.

The ^{13}C-NMR spectra of all the compounds showed signals at δ 141.1-169.5 ppm due to -C=N carbon atoms (Jung et al., 2008). The signals in the range of δ 113.1-158.2 ppm were attributed to the various aromatic ring carbon atoms.

The final proof for the compounds was obtained by recording their mass spectral analysis.

Antimicrobial screening

The *in vitro* antimicrobial activity was performed by a cup-plate method (Reddy et al., 2009; Rohini et al., 2009b). All the newly synthesized azomethines were screened for antibacterial activity against *S. aureus*, *B. subtilis*, *S. pyogenes* (Gram positive); *S. typhimurium, E. coli* and *K. pneumonia* (Gram negative) bacterial strains in addition to their antifungal activities against *A. niger, C. albicans* and *T. viridae*. For this study, ampicillin and ketoconazole were used as standards. Preliminary screening of all azomethines was performed at fixed concentrations of 1000 μg/mL. One day prior to the experiment, the bacterial cultures were inoculated in nutrient broth and incubated overnight at 37°C. The inoculated medium was poured in sterile petri dishes and allowed to solidify and set for 1 h. The cultures were swabbed on the surface of sterile nutrient agar plates using a sterile cotton swab. Similarly, one and half days prior to the experiment, the fungal cultures of *A. niger, C. albicans* and *T. viridae* were prepared in dextrose broth medium and incubated at 37°C for 36 h. Then, 50 mL of nutrient broth cultures were swabbed on the surface of sterile sabouraud dextrose agar plates. Afterwards, 6 mm wide bores were made into the agar media using a sterile cork borer and the solutions of test compounds were added into each of the bores using a sterile tip with a micropipette. Similar plates were prepared for the positive controls ampicillin and ketoconazole. The plates were allowed to stand for an hour in order to facilitate the diffusion of the drug solution. The plates were then incubated at 37°C for 24 h in the case of bacteria and at 28°C for 48-72 h in the case of fungi

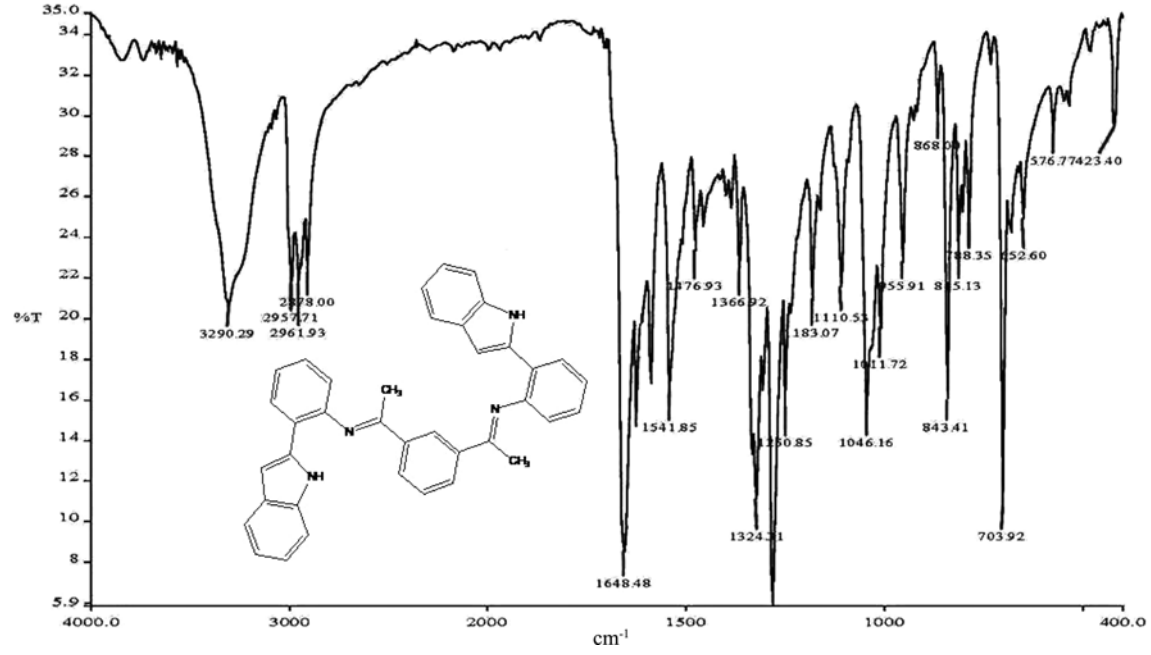


Fig. 2. IR spectrum of azomethine (Az8)

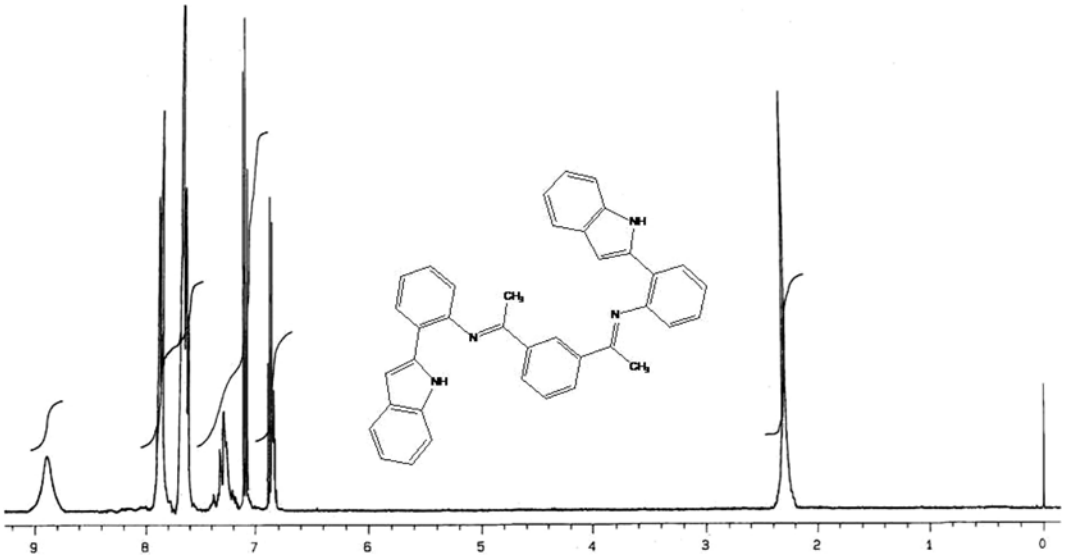


Fig. 3. ^{1}H-NMR spectrum of azomethine (Az8)

and zones of inhibition formed were measured in mm.

Zone of inhibition values are summarized in Table I. The preliminary screening data revealed that all the tested compounds Az1-13 showed moderate to good inhibition towards all tested strains. Mainly, high zone of inhibition values were observed for the Az5, Az6 azomethines for all tested strains. Furthermore, the majority of bis-azomethines (Az10-13) showed the most potent activity against all bacterial and fungal strains even against the standards.

The minimum inhibitory concentration (Mallié et al., 2005) of the potent azomethine derivatives were also verified by the liquid dilution method: the effectiveness was observed at lower concentrations. Stock solutions of test samples with 2.5, 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 μg/mL concentrations were used in this study. The minimum inhibitory concentration (MIC) which was defined no growth was recorded

Table I. Antimicrobial effects of 2-(2-arylidineaminophenyl)indoles (Az1-13) against different bacteria and fungi

Compound (1000 μg/mL)	Zone of inhibition (mm)								
	Gram-positive bacteria			Gram-negative bacteria			Fungi		
	ATCC 25923	ATCC 9372	ATCC 19615	ATCC 14028	ATCC 25922	ATCC 3882	ATCC 16404	ATCC 10231	IAM 5061
Az1	18	16	11	18	12	6	16	10	11
Az2	15	13	9	12	20	15	16	15	20
Az3	16	13	18	13	15	20	13	12	14
Az4	25	16	20	15	9	14	18	16	11
Az5	52	45	46	50	42	45	50	46	45
Az6	50	42	51	48	41	46	48	45	42
Az7	25	22	26	35	30	25	32	35	26
Az8	26	35	28	35	30	25	32	30	30
Az9	36	22	25	28	31	35	30	26	25
Az10	52	50	52	46	50	46	50	45	48
Az11	51	55	50	48	48	51	51	48	50
Az12	55	48	52	50	51	52	52	51	52
Az13	55	52	50	55	52	51	55	52	50
Std	48[a]	39[a]	35[a]	45[a]	40[a]	45[a]	45[b]	40[b]	41[b]

[a]Ampicillin, [b]Ketoconazole: *S. aureus* (ATCC 25923), *B. subtilis* (ATCC 9372), *S. pyogenes* (ATCC 19615); *S. typhimurium* (ATCC 14028), *E. coli* (ATCC 25922), *K.pneumonia* (ATCC 3882); *A. niger* (ATCC 16404); *C. albicans* (ATCC 10231) *and T. viridae* (IAM 5061)

Table II. MIC values of potent 2-(2-arylidineaminophenyl) indoles and standards against bacteria and fungi

Compound	Range of concentration (μg/mL)								
	Gram-positive bacteria			Gram-negative bacteria			Fungi		
	ATCC 25923	ATCC 9372	ATCC 19615	ATCC 14028	ATCC 25922	ATCC 3882	ATCC 16404	ATCC 10231	IAM 5061
Az5	5	10	15	5	10	10	5	15	15
Az6	10	10	5	5	15	10	10	20	20
Az10	2.5	5	2.5	10	5	5	5	20	10
Az11	5	2.5	5	5	10	2.5	5	15	5
Az12	2.5	5	2.5	2.5	2.5	2.5	2.5	5	2.5
Az13	2.5	2.5	5	2.5	2.5	5	2.5	2.5	5
Std	10[a]	20[a]	25[a]	10[a]	15[a]	10[a]	15[b]	25[b]	20[b]

[a]Ampicillin, [b]Ketoconazole: *S. aureus* (ATCC 25923), *B. subtilis* (ATCC 9372), *S. pyogenes* (ATCC 19615); *S. typhimurium* (ATCC 14028), *E. coli* (ATCC 25922), *K.pneumonia* (ATCC 3882); *A. niger* (ATCC 16404); *C. albicans* (ATCC 10231) *and T. viridae* (IAM 5061)

(Table II). Comparison of MIC values (in μg/mL) of potent indole azomethines and standards against different strains are presented in Fig. 1. From this study, it is clear that Az5, Az6 compounds (at MIC range 5-20 μg/mL) and especially the bis indole azomethines (Az10-13) (MIC range 2.5-20 μg/mL) showed superior activity towards all bacterial and fungal strains.

In conclusion, we have synthesized thirteen novel indole azomethines by using 2-(o-aminophenyl)indole and diketones (R-CO-R^{l}/R-CO-X-CO-R^{l}). In fact, while several synthetic routes have been published for a variety of azomethines, there have been no attempts to synthesis a mono and bis-2-(2-arylideneaminophenyl)indole azomethines; antimicrobial studies have also not been reported. The compounds which contain pyridyl substituents and two azo (-C=N) groups in their structures (bis-azomethines) seem to be more potent even compared to standards like ampicillin and ketoconazole. However, the method of action of these compounds is unknown. These observations may promote an additional research in this field. Further development of this group of compounds may lead to compounds with better pharmacological profiles than the standards and serve as templates for the construction of better drugs to combat bacterial and fungal infections.

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