



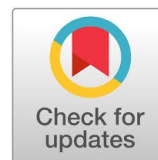
Original Research Article

Synthesis and anti-microbial activities of azomethine and aminomethyl phenol derivatives

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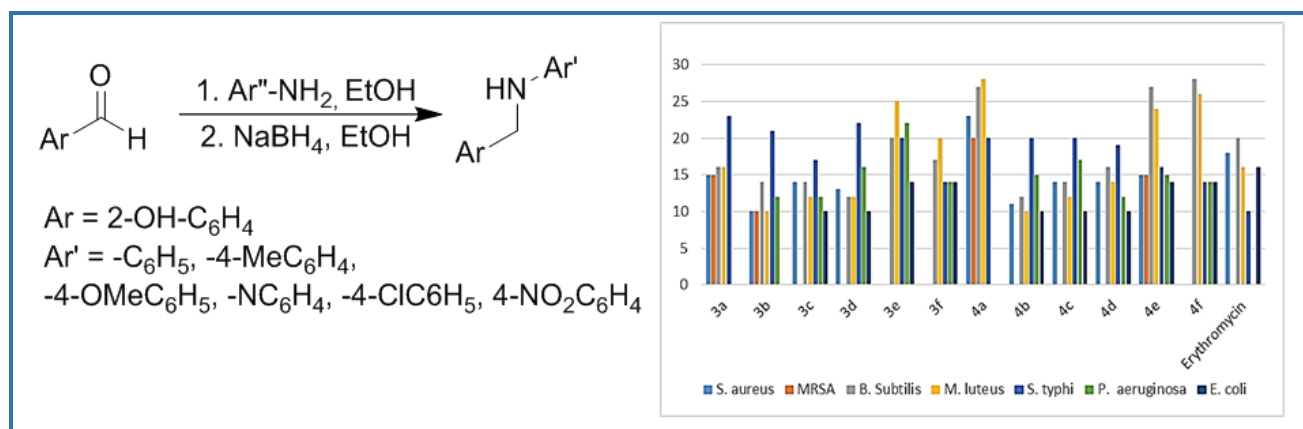
Aldehyde
Azomethines
Aminomethyl phenol
Antibacterial activity
Antifungal activity

ABSTRACT

A series of azomethine and aminomethyl phenol derivatives was synthesized, and characterized using mass, IR, and NMR spectral techniques. *In vitro* antimicrobial activities of the compounds were evaluated against different gram-positive and gram-negative bacterial and fungal strains by measuring zone of inhibition using agar diffusion method. Results of the antimicrobial screening indicated that the compound **4a** was the most active antimicrobial agent (100 µg/mL). The compounds **3a**, **4e**, **4f** were exhibited best *in vitro* anti-microbial activity against the gram positive bacterial strains such as *Bacillus subtilis*, *Micrococcus luteus* and gram negative bacterial strain *Salmonella typhi*, and fungal strain *Candida albicans*.

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Graphical Abstract



Introduction

The development of newer antibacterial and fungal drugs is necessary as the pathogens evolve resistance against the available drugs. Therefore, the synthesis of new and effective antimicrobial drugs become very important as many research programs have been directed toward the design of newer agents. Azomethines have potential for both chemical and biological activity [1–5], which is due to the presence of carbon nitrogen double bond. Azomethine and aminomethyl phenol derivatives represent one of the most biologically active classes of compounds, possessing a wide spectrum of activities such as antihyperglycemic [6], estrogenic and cytotoxic activities [7–9], anticancer [10], diuretic [11], and antiparasitic [12] activities. However, very limited literatures are available for the antimicrobial activities of aminomethyl phenol derivatives [13].

In this work, we synthesized series of azomethine and aminomethyl phenol derivatives possessing different substituents. The antimicrobial activities of the synthesized material was also assessed. The results showed that most of the synthesized molecules exhibited potent antimicrobial activity.

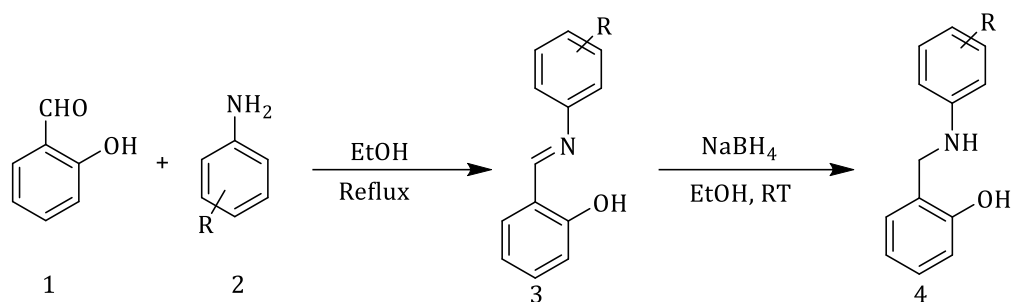
Experimental

General procedure for the synthesis of azomethines

8.19 mmol of aniline, 10 mL of dry ethanol, and 8.19 mmol of salicylaldehyde were mixed at 0 °C. The mixture was stirred for 10 min. Then, it was heated upto 70 °C and kept for 6 h. The progress of the reaction was monitored by TLC, after the complete conversion of starting materials, the reaction mixture was cooled down to room temperature and formed precipitate was filtered off. Then it was washed with chilled ethanol (2×10 mL) and dried under vacuum. The crude material was recrystallized using hot ethanol, affording the desired product as a solid.

2-((E)-(phenylimino)methyl)phenol 3a

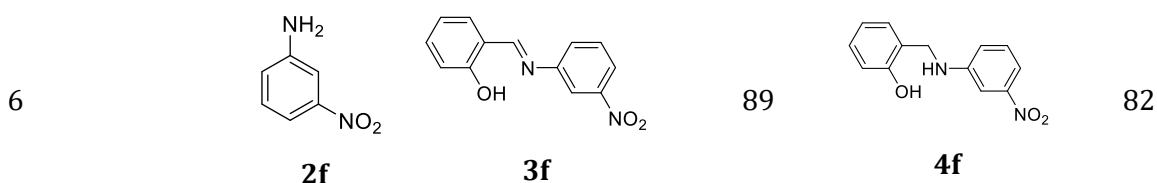
Pale yellow crystals, yield 87%, mp 51–52 °C, IR (KBr) (ν_{max} / cm^{-1}): 3436, 3054, 1615, 1483, 1274, 896 and 754. ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 13.07 (s, 1H), 8.94 (s, 1H), 7.64 (d, $J=7.6$ Hz, 1H), 6.99–6.94 (m, 2H), 7.30–7.27 (m, 1H), 7.65–7.31 (m, 5H). ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$): δ 164, 160.7, 148.5, 133.7, 133, 129.9, 127.4, 121.8, 119.7, 119.5, 117. MS/APCI (m/z): 198 ($\text{M}^+ + \text{H}$).



Scheme 1. synthesis of azomethines **3** and their aminomethyl phenols **4**.

Table 1. Preparation of azomethines **3** and their aminomethyl phenols **4**.

S.No ^a	Aldehyde	Amine 2	Product 3	Yield of 3 (%) ^b	Product 4b	Yield of 4 (%) ^b
1				87		80
		2a	3a		4a	
2				94		91
		2b	3b		4b	
3				91		83
		2c	3c		4c	
4 ^c				91		84
	1	2d	3d		4d	
5				84		76
		2e	3e		4e	



^aTo the formed azomethine (1 eq) in dry ethanol (10 vol) sodium borohydride (1.0 eq) was added in portions over a period of 20 min at 0 °C under N₂ atmosphere. To the formed azomethine (1 eq) in dry ethanol (10 vol) sodium borohydride (1.0 eq) was added in portions over a period of 20 min at 0 °C under N₂ atmosphere

^bThe yields are of isolated yields. ^cThe reaction was carried out using diamine (4.62 mmol) and salicylaldehyde (18.49 mmol)

General procedure for the synthesis of aminomethyl phenol

To a stirred suspension of the imine (1 eq) in dry ethanol (10 vol), sodium borohydride (1 eq) was added in portions over a period of 20 min at 0 °C under N₂ atmosphere. Then, the suspension was dissolved to get clear solution. The reaction mixture was kept at 0 °C for 2 h. The progress of the reaction was monitored by TLC, after the complete conversion of starting materials, the white solid was filtered off. The white solid was washed with chilled ethanol (2×10 mL) and dried under vacuum. The crude material was recrystallized by using hot ethanol, affording the desired product as white solid.

2-(phenylaminomethyl) phenol 4a

White solid, yield 80%, mp 107–108 °C [21–22], IR (KBr) (ν_{max} / cm⁻¹): 3640, 3265, 2865, 1300, 1251, 934 and 754. ¹H NMR (300 MHz, DMSO-*d*₆): δ 7.14–6.96 (m, 5H), 6.78–6.44 (m, 4H), 4.14 (s, 2H); ¹³C NMR (75 MHz, DMSO-*d*₆): δ 156, 149.3, 129.2, 128.5, 127.8, 126.2, 118.4, 115.9, 115.4, 122.6, 41.9. MS/APCI (*m/z*): 200 (M⁺+H).

Results and Discussion

The azomethine [14–20] and aminomethyl phenol derivatives [21–24] were synthesized by following a well-established protocol. The protocol is shown in Scheme 1. These derivatives were designed in such a way to have electron releasing and electron withdrawing groups in the amine part. The aminomethyl phenols **4** were prepared in large quantities through condensation of salicylaldehyde **1** with substituted anilines **2** (84–94% yield) and subsequent reduction of the imine **3** using sodium borohydride-ethanol reagent system. The obtained yields are presented in Table 1.

All the compounds were purified by recrystallization from ethanol. All the products were characterized using MS, IR, ¹H NMR, and ¹³C NMR spectral techniques. It was reported in the literature

that the *E* isomer of imine **3** was formed [21–24] possibly due to the intramolecular *H*-bonding between hydroxyl group and nitrogen atom [25].

The results in Table 1 clearly show that all of the reactions gave the desired products **3a–f** and **4a–f** in good to excellent yields. It was observed that the anilines possessing an electron-releasing group gave higher yields than those with an electron-withdrawing group.

In the NMR spectra, all of the compounds exhibited characteristic signals appropriately (See experimental section). For example, in the IR spectrum, **3a** strong absorption at 1615 cm⁻¹ corresponds to the stretching vibration of the C=N group and 3336 cm⁻¹ which relates to the OH group. In the ¹H NMR spectrum of **3a**, singlets at δ 13.07 and 8.94 ppm correspond to -HC=N- and -OH protons, respectively. The downfield shift observed in -HC=N- proton is due to the strong electronegativity of nitrogen. Meanwhile, the mass spectrum (MS/APCI) of **3a–f** display a molecular ion peak at *m/z* corresponding to (M+H). In the case of **4a**, a strong absorption at 3640 cm⁻¹ corresponds to the stretching vibration of the -OH group and that at 3265 cm⁻¹ relates to the stretching of -NH group. A singlet δ 4.14 ppm observed in the ¹H NMR spectrum of **4a** corresponds to the benzylic proton. Meanwhile, the mass spectrum (MS/APCI) of **4a–f** display a molecular ion peak at *m/z* (M+H).

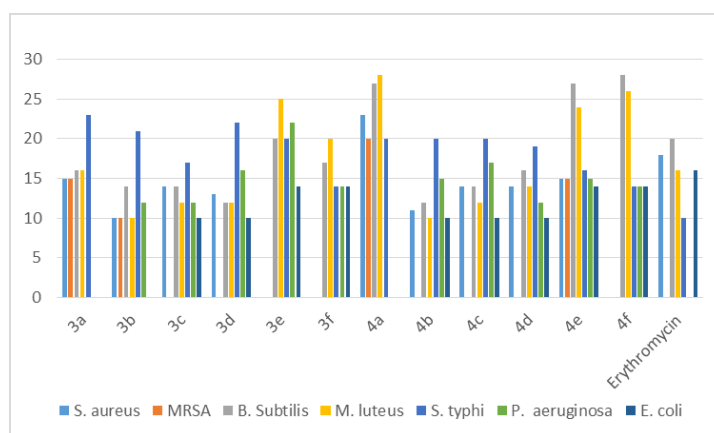
Antibacterial activity

Biological activities of the azomethines and aminomethyl phenols were screened for antibacterial activity against four gram-positive bacterial strains including, *Staphylococcus aureus*, Methicillin resistant *Staphylococcus aureus* (MRSA), *Bacillus subtilis*, and *Micrococcus luteus* and three gram-negative pathogens *Salmonella typhi*, *Pseudomonas aeruginosa*, and *Escherichiacoli*. The biological activities were screened by Kirby-Bauer method [26], using disc diffusion technique at a concentration of 100 μ g/mL with erythromycin as a standard drug. The zone of inhibition is compared in Table 2 and the corresponding clustered column chart is demonstrated in Figure 1. In general, a very good antibacterial activity was observed in all the compounds on the chosen microorganism.

According to the results of the antibacterial activity screening, all the synthesized compounds possess excellent antibacterial activity against two gram-positive bacterium *Bacillus subtilis*, and *Micrococcus luteus*, and one gram-negative bacterium viz. *Salmonella typhi*. The compounds **3a**, **3b**, **4a**, and **4e** were found to possess very good activity against *Staphylococcus aureus*, Methicillin resistant *staphylococcus aureus* (MRSA), *Bacillus subtilis*, *Micrococcus luteus* and *Salmonella typhi*. Azomethines **3c**, **3d**, and **3f**, aminomethyl phenols **4b**, **4c**, **4d** and **4f** have showed moderate to good

Table 2. Antibacterial activity of azomethines **3a-f** and aminomethylphenols **4a-f** against bacterial strains.

Compds	Zone of Inhibition in mm						
	Gram Positive				Gram Negative		
	<i>S. aureus</i>	<i>MRSA</i>	<i>B. Subtilis</i>	<i>M. luteus</i>	<i>S. typhi</i>	<i>P. aeruginosa</i>	<i>E. coli</i>
3a	15	15	16	16	23	-	-
3b	10	10	14	10	21	12	-
3c	14	-	14	12	17	12	10
3d	13	-	12	12	22	16	10
3e	-	-	20	25	20	22	14
3f	-	-	17	20	14	14	14
4a	23	20	27	28	20	-	-
4b	11	-	12	10	20	15	10
4c	14	-	14	12	20	17	10
4d	14	-	16	14	19	12	10
4e	15	15	27	24	16	15	14
4f	-	-	28	26	14	14	14
Erythromycin	18	-	20	16	10	-	16

**Figure 1.** Clustered column chart for the antibacterial activity of azomethines **3a-f** and aminomethylphenols **4a-f** against bacterial strains

antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Micrococcus luteus* and *Salmonella typhi*. *Pseudomonas aeruginosa*, and *Escherichia coli*.

Antifungal activity

The microbial organism *Candida albicans* is a fungal pathogen, causing wide range of diseases in susceptible persons [27]. These may be superficial infections to severe life threatening infections involving many essential organs. Recently, there has been a considerable increase in the incidence of disease attributable to *Candida albicans*, with the spread of AIDS, the widespread use of immuno-suppressive therapy and prolonged survival of patients with critical illnesses [28]. Conventional therapy for the control of fungal infections relies on the use of ketoconazole drug. However, the emergence of *C. albicans* which isolates resistances to these drugs has serious implications for the continued success of these prescription anti-fungal compounds [19, 20]. In the present study, antifungal activity of the synthesized azomethines **3a-f** and aminomethyl phenols **4a-f** were investigated against five fungal pathogens viz. *Aspergillus niger*, *Fusarium oxysporum*, *Penicillium sp.*, *Candida albicans*, and *Candida tropicalis*. The disc diffusion technique was followed using Kirby-Bauer method [26], at a concentration of 100 µg/mL with ketoconazole as standard drug, and the obtained results are summarized in Table 3. The clustered column chart is revealed in Figure 2, indicating the fact that all the compounds have excellent antifungal activity against *Candida albicans* species in particular. The compounds **3a** and **4a** have shown very good antifungal activity against all the five screened organisms. The azomethines **3c** and **3e** and the aminomethyl phenols **4b-e** have exhibited moderate antifungal activity against *Aspergillus niger*. The compounds **3c** and **3d** have shown significant antifungal activity against *Penicillium sp.* Also, the azomethine derivative **3e** and the aminomethylphenol derivatives **4d** and **4e** which have shown good activity against *Candida tropicalis*.

Table 3. Antifungal activity of azomethines **3a-f** and aminomethylphenols **4a-f** against bacterial strains.

Compds	Zone of Inhibition in mm				
	<i>Aspergillus niger</i>	<i>Fusarium oxysporum</i>	<i>Penicillium sp.</i>	<i>Candida albicans</i>	<i>Candida tropicalis</i>
3a	10	16	10	15	13
3b	-	-	-	10	15
3c	12	-	10	10	8
3d	-	-	10	17	15
3e	13	-	-	34	-
3f	-	-	-	17	-
4a	25	15	17	28	24

4b	12	-	-	10	-
4c	12	-	-	10	-
4d	10	-	-	25	13
4e	11	-	-	27	15
4f	-	-	-	20	-
Ketoconazole	-	-	-	24	-

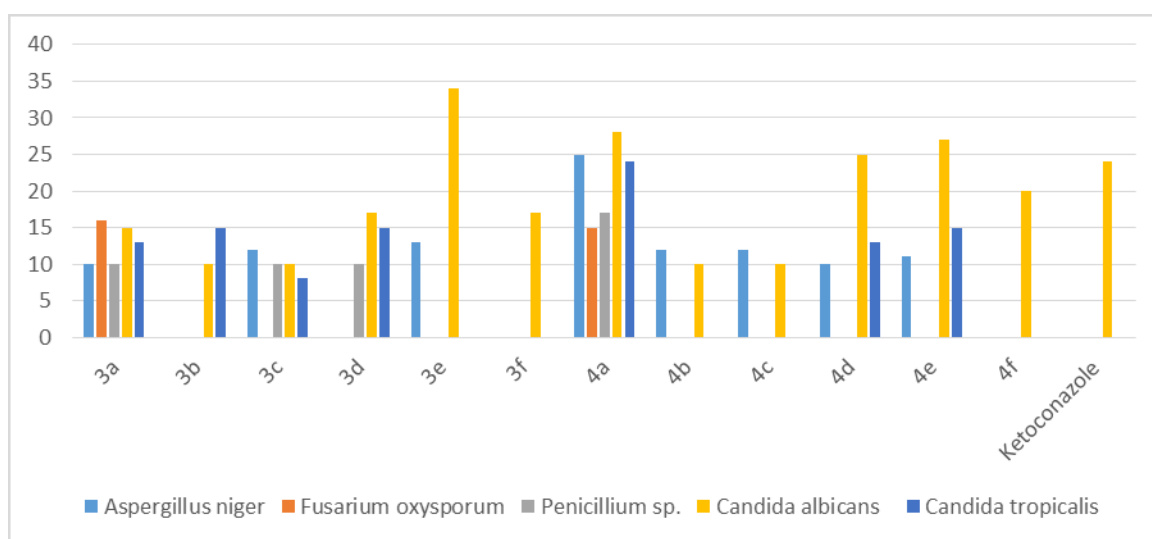


Figure 2. Clustered column chart for the antifungal activity of azomethines **3a-f** and aminomethylphenols **4a-f** against bacterial strains.

Conclusion

In this study, we have synthesized series of azomethine and aminomethyl phenol derivatives in good to excellent yields using mass, IR, ^1H NMR and ^{13}C NMR spectral techniques. In vitro antimicrobial activities of all the synthesized compounds **3a-f** and **4a-f** were tested at a concentration of 100 $\mu\text{g/mL}$. Among the synthesized compounds, the derivative **4a** exhibited the best antimicrobial activity against gram positive bacterial strain such as *S. aureus*, *MRSA*, *Bacillus subtilis*, *Micrococcus luteus* and gram negative bacteria of *Salmonella typhi*. The aminomethyl phenol derivatives (**4e** and **4f**) containing electron withdrawing group were found to be the best in vitro anti-microbial activity against the gram positive bacterial strains such as *Bacillus subtilis*, *Micrococcus luteus* and gram negative bacterial strain *Salmonella typhi*, and fungal strain *Candida albican*.

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Disclosure Statement

No potential conflict of interest was reported by the authors.

Supporting Information

Additional supporting information related to this article can be found, in the online version, at [10.33495/SAMI/AJGC/2019.4.7](https://doi.org/10.33495/SAMI/AJGC/2019.4.7).

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