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Bioactivity of azomethines derived mechanochemically from 2-amino pyridine and studies on the effect of substituents on the reaction

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Azomethines with pyridine framework serve as excellent pharmacophore. A number of azomethine derivatives were synthesised from 2-aminopyridine and differently substituted aromatic aldehydes in excellent to almost quantitative yields through green, mechanochemical protocol. Influence of the substituents in the nuclei of aromatic aldehydes on the rate of the reaction was investigated. Presence of ortho hydroxy groups in the nucleus of aromatic aldehydes led to the completion of the reactions in almost no time with nearly quantitative yields. 2,2′-diphenyl-1-picrylhydrazyl (DPPH) radical was used to evaluate the *in vitro* antioxidant activity of the prepared azomethines and the results were compared with standard natural antioxidant L-ascorbic acid. Most of the derivatives showed fairly strong antioxidant property. Antibacterial activity of the prepared azomethines were examined against *Bacillus subtilis* (Gram-positive) and *Escherichia coli* (Gram-negative) strains by using agar well diffusion method. Some of the azomethines exhibited encouraging antibacterial activities.

Keywords: Azomethine, mechanochemistry, DPPH, antibacterial activity.

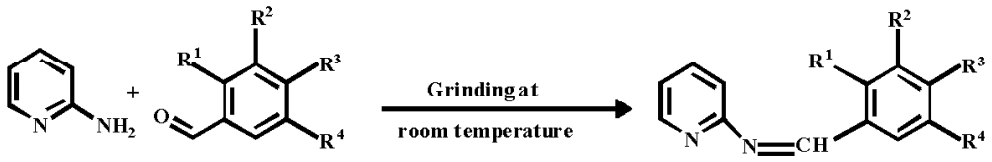
Introduction

Methodologies for the formation of azomethines are attractive to synthetic chemists due to occurrence of this structural unit in a variety of naturally and pharmacologically important molecules[1–3]. Diverse broad-spectrum biological activities like anti-proliferative[4–8], anti-inflammatory[9–13], analgesic[10–14], antimicrobial[15–19], antiviral[20,21], anticonvulsant[22], antifungal[23–25], antimalarial[26], antileishmanial[27–29], antitubercular[10,30], antibacterial[2,25,31,32], antioxidant[9,25,33], anthelmintic[34] etc. prevail in the properties of azomethines. Some important pharmacologically active molecules containing azomethine unit include an cistrocladidine[35], chitosan-derived Schiff base[36], *N*-(salicylidene)-2-hydroxyaniline[37,38]. Azomethines are also good Mannich electrophiles and excellent heterodienophiles for the construction of six-membered nitrogen heterocycles[39,40]. Conventional methods for the formation of azomethines involve condensation between a carbonyl compound and a primary amine in a suitable solvent usually at its boiling point. Addition of a small amount of acid is often necessary to enhance electrophilicity of the carbonyl carbon to hasten the reaction. Long reaction time, low yield of the product and cost to attain the appropriate temperature for the reaction and evaporation of the solvent during isolation of the product cripple this conventional procedure from economic and environmental point of view. In recent years mechanochemistry has emerged as an efficient tool for the performance of reactions under solvent-free conditions[41–45]. Azomethines with pyridine framework frequently appear in agrochemicals and pharmaceuticals[46–49]. Employment of mechanochemistry and studies on the effects of substituents on the course of reaction, however, have not been found for the synthesis of azomethines from 2-aminopyridine and various aromatic aldehydes to the best of our knowledge. Our present study is, therefore, mainly focused on mechanochemical synthesis of azomethines from aromatic aldehydes and 2-amino pyridine and finding out the effects of substituents on the rate of the reaction and yield of the products. In addition to the synthetic aspects a detailed study of the radical scavenging and antimicrobial activity of the prepared azomethines is also presented.

Results and discussion

Chemistry:

Success of the reaction between a primary amine and an aldehyde classically depends on the electrophilicity and nucleophilicity of the carbonyl and amine counterparts respectively and the steric crowding around the electrophilic and nucleophilic sites. To examine the influence of the polar effects of the substituents on the rate of the reaction we have used aromatic aldehydes with electron attracting (entries **2**-**6**) as well as electron releasing (entries **7**-**10**) groups in the nucleus (Scheme 1). For the study of steric effects aromatic aldehydes with ortho substituents (entries **11**-**15**) with respect to the formyl group were used. Yields for all the reactions were excellent but surprisingly time needed for completion of the reactions were found to be almost insensitive to the electronic nature of the ring substituents. In contrast presence of a hydroxy function ortho to the formyl group dramatically accelerated the reaction despite the apparent involvement of the steric factor caused by the ortho substituent(s). It is worth mentioning that mechanochemical reaction between 3-ethoxysalicylaldehyde and a number of aromatic amines also led to almost quantitative yield of the corresponding azomethine in very short time although no investigation of the influence of substituents on the course of the reaction and evaluation of bioactivity of the prepared azomethines were found in this paper[44]. In the present case also yields for the reactions with aromatic aldehydes containing ortho hydroxy groups were nearly quantitative. In the neat phase, motion of reactant molecules is restricted and so further decrease in entropy during the formation of the transition state is much lower than that for solution phase reactions. So these



Entry	R^1	R^2	R^3	R^4	Time (min)	Yield (%)[a]	m.p. (°C)
1	H	H	H	H	30	86	95
2	NO_2	H	H	H	40	78	128
3	H	NO_2	H	H	40	80	76
4	H	H	NO_2	H	45	87	123
5	H	H	Cl	H	60	82	85
6	H	H	Br	H	70	75	76
7	H	H	CH_3	H	40	82	122
8	H	H	OCH_3	H	45	84	158
9	H	H	$N(CH_3)_2$	H	40	78	68
10	H	OCH_3	OH	H	45	86	138
11	OH	H	H	H	10	96	64
12	OH	OCH_3	H	H	5	98	82
13	OH	H	OCH_3	H	2	97	72
14	OH	OC_2H_5	H	H	3	98	94
15	OH	OCH_3	H	Br	2	98	92

[a]yields refer to the yields after crystallisation.

Scheme 1. Solvent free mechanochemical synthesis of 2-aminopyridine derived azomethines.

solid phase reactions may be referred to as entropy assisted reactions. Hydrogen bonding between the ring nitrogen of 2-aminopyridine and the phenolic hydrogen of aromatic aldehydes may favourably dispose reactant molecules for nucleophilic attack at the carbonyl carbon by the amino nitrogen. Stabilization of the transition state through intramolecular hydrogen bonding which is further strengthened by development of partial negative charge on carbonyl oxygen is speculated to decrease the free energy of activation for the reaction making it kinetically favourable (Fig. 1). Strong intramolecular hydrogen bonding as evidenced by extremely high downfield shift (δ ~ 14 ppm) of the phenolic proton and insensitivity of the O-H stretching frequency to dilution, may be responsible for the thermodynamic stability of these azomethines leading to almost quantitative yields for them.

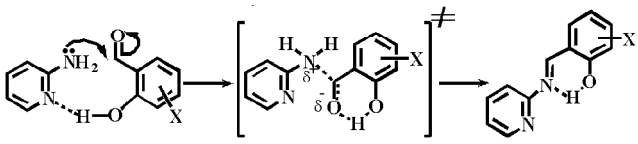


Fig. 1. Proposed mechanistic pathway for reaction between 2-aminopyridine and aromatic aldehydes containing ortho hydroxy groups.

Biological activity:

In vitro DPPH radical scavenging activity:

Biological properties of azomethines are often attributed to the presence of unshared pair of electrons on imino nitrogen. Capacity of the azomethines to undergo facile chelation plays a vital role in their antioxidant activities[50]. The ability of the prepared azomethine derivatives to scavenge DPPH radical was evaluated and expressed in terms of IC_{50} values (Table 1). The assay is based on the estimation of the electron or hydrogen transfer ability of compounds to the DPPH radical to form the corresponding hydrazine and the activity of each compound depends on the stabilization of the resulting free radical species[51].

The varied range of data presented in Table 1 and Fig. 2 could establish the moderate to strong antioxidant property of some of the azomethine derivatives, which indicates their radical scavenging as well as reducing abilities. Compound **3** displayed very poor activity when compared to the standard antioxidant L-ascorbic acid and the compound **4** was found to be inactive in this series. It could not scavenge 50% of the DPPH radicals even at the highest tested concentration (100 μM) [Fig. 2]. Significantly, the compounds having phenolic -OH group (**10**, **11**,**12**,**13**,**14** and**15**) exhibited relatively higher activities compared to the other analogues, suggesting that the substituents present in the respective aromatic ring have a profound influence on the antioxidant ac-

Table 1. *In vitro* DPPH radical scavenging activity of azomethine derivatives

Compounds	IC_{50} (μM) ± S.E.
1	62.17±2.63
2	54.59±1.52
3	80.71±0.63
4	> 100
5	61.16±1.43
6	42.82±1.17
7	38.98±0.51
8	46.27±1.85
9	53.51±1.35
10	30.34±1.74
11	18.48±1.53
12	15.24±0.88
13	27.03±1.81
14	20.46±1.28
15	24.76±1.02
L-Ascorbic acid	8.44±0.78

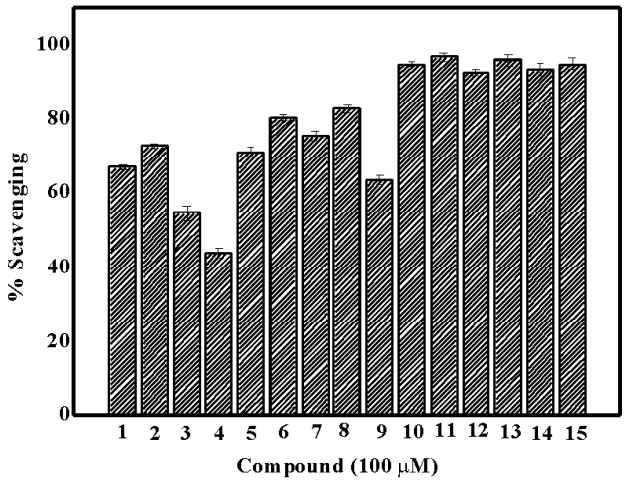


Fig. 2. *In vitro* DPPH radical scavenging activity of azomethine derivatives (100 μM). Error bars represent the standard error in each compound of three separate determinations.

Table 2. Antibacterial activity (diameter of inhibition zone[a] in mm) of azomethine derivatives

Compound ↓	Gram-positive bacteria (*Bacillus subtilis*)						Gram-negative bacteria (*Escherichia coli*)					
Concentration → (in mg/ mL)	0.05	0.1	0.2	0.3	0.4	0.5	0.05	0.1	0.2	0.3	0.4	0.5
1	–	–	–	–	–	–	–	–	–	–	–	–
2	–	–	–	–	–	–	–	–	–	–	–	–
3	–	–	–	–	–	–	–	–	–	–	–	–
4	–	–	–	–	–	–	–	–	–	–	–	–
5	–	–	–	–	–	–	–	–	–	–	–	–
6	–	–	–	–	–	–	–	–	–	–	–	–
7	–	–	–	–	–	–	–	–	–	–	–	–
8	–	8	9	10	11	14	–	–	–	–	–	–
9	–	–	–	–	–	–	–	–	–	–	–	–
10	8	10	11	11	14	16	–	–	–	–	–	–
11	11	12	14	14	17	19	–	9	10	12	15	16
12	12	12	14	14	18	20	7	8	10	11	14	19
13	12	15	18	19	19	20	8	8	10	16	17	20
14	14	17	19	20	22	22	10	10	12	21	25	30
15	11	12	15	17	21	23	8	8	10	11	14	18
Ampicillin (0.1 mg/mL) [Positive control]		24					20					
DMSO [Vehicle control]			Not detected						Not detected			

[a]Including the diameter of well (5 mm); – No activity (diameter of the inhibition zone less than 7 mm).

tivity by enhancing their ability of hydrogen donation. Generally phenolic compounds act as potent antioxidants due to the formation of stable phenoxyl radical through the abstraction of hydrogen atom by DPPH radical[52,53].

Antibacterial activity:

In the present study, antibacterial activity of all the synthesised azomethines were evaluated against one Gram-positive and one Gram-negative strains over a range of concentrations (0.05–0.5 mg/mL) by agar well diffusion method. The results of bactericidal assessments were expressed in terms of diameter of the inhibition zones in mm and summarized in Table 2.

The solvent vehicle DMSO, was found to be inactive against all the tested bacteria. At 0.1 mg/mL concentration, the positive control ampicillin responded to a significant level of bacterial inhibition against both of these Gram-positive and Gram-negative organisms.

The prepared azomethine derivatives displayed a broad spectrum of antibacterial activity against both the microorganisms in a concentration dependent manner. It is noteworthy that all the tested bacteria were highly susceptible to inhibition by the compounds (**11**, **12**, **13**, **14** and **15**) derived from the aldehydes having ortho hydroxy group. Compound **14**, the azomethine analogue prepared from 3-ethoxy salicylaldehyde exhibited remarkable activity against both the bacteria. It was observed that the growth of Gram-negative bacteria viz. *E. coli* was strongly inhibited at the higher doses (0.3–0.5 mg/mL) of compound **14** (Fig. 3). In fact, at these concentrations this compound was found to show a greater potency in comparison to the standard drug ampicillin (0.1 mg/mL). The efficacy of azomethine **13** (0.5 mg/mL), derived from 4-methoxy salicylaldehyde, was nearly equivalent to that exhibited by 0.1 mg/mL of ampicillin. It has been mentioned earlier that azomethine pharmacophores are considered as a promising class of bioactive compounds and the imino ni-

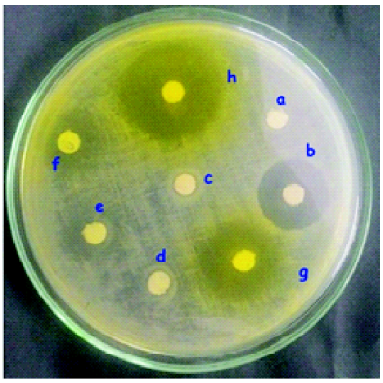


Fig. 3. Antibacterial activity of compounds against *Escherichia coli*. a: DMSO (vehicle control); b: Ampicillin (0.1 mg/mL; positive control); c: 0.05 mg/mL; d: 0.1 mg/mL; e: 0.2 mg/mL; f: 0.3 mg/mL; g: 0.4 mg/mL; h: 0.5 mg/mL of compound **14**.

trogen plays a crucial role in several biochemical reactions[50]. Presumably, involvement of the nitrogen atom of azomethine moiety in the formation of a hydrogen bond with the active centers of cell constituents results in interferences in normal cellular processes[31,54]. Presence of additional hydrogen bonding site viz. the hydroxy function in azomethines with hydroxylated aromatic nuclei, may therefore, be expected to exhibit interesting biological activity.

The compounds **8** and **10** with *p*-anisaladehyde and vanillin templates respectively in the aldehyde counterpart of azomethines showed moderate activity towards the Gram-positive strain (*Bacillus subtilis*). However, they were found to be inactive against Gram-negative bacteria (*Escherichia coli*). The rest of the compounds did not show any activity towards all the bacteria used in this study.

Experimental

Chemistry

General Information:

All the necessary chemicals were procured from Spectrochem, Merck or Sigma-Aldrich. Thin layer chromatography (TLC) using silica gel GF254 plates was used to monitor the reactions. Iodine vapour staining was used for the location of spots on TLC. Electrical melting point apparatus (S.I.) was used to determine the melting points and these are uncorrected. IR spectra were recorded on KBr pellets in a Perkin-Elmer 1330 apparatus. Recording of ^{1}H NMR spectra was done on a Bruker 300 NMR (300 MHz) spectrometer using $CDCl_3$ as solvent and tetramethylsilane (TMS) as internal standard. Perkin-Elmer instrument 2400 Series II CHN analyzer was used to carry out the elemental analyses.

*Preparation of azomethines (entries **1**-**15**):*

A mixture of 2-aminopyridine (1 mmol) and appropriate aromatic aldehyde (1 mmol) was ground thoroughly in a clean, dry porcelain mortar with a pestle until the mixture became sticky. The sticky mass was left in air for 2–70 min (Table 1) with a little grinding from time to time. When the reaction was completed (as indicated by TLC), the solid mass was taken out of the inner wall of the morter with the flat end of a spatula. All the compounds were recrystallised from dilute aqueous ethanol.

*N-Benzylidenepyridin-2-amine (**1**):* Yield 86%, m.p. 95°C; Anal. Calcd. for $C_{12}H_{10}N_2$; C, 79.10; H, 5.53; N, 15.37. Found: C, 78.93; H, 5.39; N, 15.26%; IR (KBr) ν_{max}: 1490, 1580, 1610, 3060 cm^{-1}; ^{1}H NMR ($CDCl_3$): 6.65–6.85 (2H, m, Ar***H***), 7.17–7.46 (2H, m, Ar***H***), 7.75 (1H, m, Ar***H***), 7.93–8.10 (3H, m, Ar***H***), 8.51 (1H, m, Ar***H***), 9.15 (1H, s, N=C***H***).

*N-(2-Nitrobenzylidene)pyridin-2-amine (**2**):* Yield 78%, m.p. 128°C; Anal. Calcd. for $C_{12}H_9N_3O_2$; C, 63.43; H, 3.99; N, 18.49. Found: C, 63.26; H, 3.82; N, 18.33%; IR (KBr) ν_{max}: 1375, 1485, 1502, 1575, 1612, 3070 cm^{-1}; ^{1}H NMR ($CDCl_3$): 6.87 (1H, m, Ar***H***), 7.56–7.60 (2H, m, Ar***H***), 7.73 (1H, m, Ar***H***), 7.95–8.12 (2H, m, Ar***H***), 8.21 (1H,m, Ar***H***), 8.51 (1H, m, Ar***H***), 9.12 (1H, s, N=C***H***).

*N-(3-Nitrobenzylidene)pyridin-2-amine(**3**):* Yield 80%, m.p. 76°C; Anal. Calcd. for $C_{12}H_9N_3O_2$; C, 63.43; H, 3.99; N, 18.49. Found: C, 63.32; H, 3.85; N, 18.26%; IR (KBr) ν_{max}: 1375, 1495, 1510, 1585, 1615, 3075 cm^{-1}; ^{1}H NMR ($CDCl_3$): 6.95 (1H, m, Ar***H***), 7.58 (1H, m, Ar***H***), 7.77 (1H, m, Ar***H***), 7.85 (1H, m, Ar***H***), 8.22–8.35 (3H, m, Ar***H***), 8.51 (1H, m, Ar***H***), 9.12 (1H, s, N=C***H***).

*N-(4-Nitrobenzylidene)pyridin-2-amine (**4**):* Yield 87%, m.p. 123°C, Anal. Calcd. for $C_{12}H_9N_3O_2$; C, 63.43; H, 3.99; N, 18.49. Found: C, 63.34; H, 3.89; N, 18.38%; IR (KBr) ν_{max}: 1387, 1496, 1510, 1580, 1640, 3055 cm^{-1}; ^{1}H NMR ($CDCl_3$): 7.12 (1H, m, Ar***H***), 7.57 (1H, m, Ar***H***), 7.76 (1H, m, Ar***H***), 7.93 (2H, d, *J* 8.8 Hz, Ar***H***), 8.27 (2H, d, *J* 8.8 Hz, Ar***H***), 8.51 (1H, m, Ar***H***), 9.14 (1H, s, N=C***H***).

*N-(4-Chlorobenzylidene)pyridin-2-amine (**5**):* Yield 82%, m.p. 85°C; Anal. Calcd. for $C_{12}H_9N_2Cl$; C, 66.52; H, 4.19; N, 12.93. Found: C, 66.39; H, 4.09; N, 13.02%; IR (KBr) ν_{max}: 698, 1500, 1570, 1612, 3060 cm^{-1}; ^1H NMR ($CDCl_3$): 6.98 (1H, m, Ar***H***), 7.47 (1H, m, Ar***H***), 7.63 (2H, d, *J* 8.8 Hz, Ar***H***), 7.72 (1H, m, Ar***H***), 7.91 (2H, d, *J* 8.8 Hz, Ar***H***), 8.51 (1H, m, Ar***H***), 9.14 (1H, s, N=C***H***).

*N-(4-Bromobenzylidene)pyridin-2-amine (**6**):* Yield 75%, m.p. 76°C; Anal. Calcd. for $C_{12}H_9N_2Br$; C, 55.20; H, 3.47; N, 10.73. Found: C, 55.08; H, 3.38; N, 10.65%; IR (KBr) ν_{max}: 626, 1510, 1590, 1610, 3050 cm^{-1}; ^1H NMR ($CDCl_3$): 6.83 (1H, m, Ar***H***), 7.29 (1H, m, Ar***H***), 7.51 (2H, d, *J* 8.8 Hz, Ar***H***), 7.70 (1H, m, Ar***H***), 7.82 (2H, d, *J* 8.8 Hz, Ar***H***), 8.51 (1H, m, Ar***H***), 9.16 (1H, s, N=C***H***).

*N-(4-Methylbenzylidene)pyridin-2-amine (**7**):* Yield 82%, m.p. 122°C; Anal. Calcd. for $C_{13}H_{12}N_2$; C, 79.56; H, 6.16; N, 14.27. Found: C, 79.43; H, 6.08; N, 14.18%; IR (KBr) ν_{max}: 1505, 1600, 1614, 2980, 3080 cm^{-1}; ^1H NMR ($CDCl_3$): 2.19 (3H, s, -C***H***$_3$), 6.89 (1H, m, Ar***H***), 7.09 (2H, d, *J* 8.8 Hz, Ar***H***), 7.45 (1H, m, Ar***H***), 7.75 (1H, m, Ar***H***), 7.89 (2H, d, *J* 8.8 Hz, Ar***H***), 8.51 (1H, m, Ar***H***), 9.16 (1H, s, N=C***H***).

*N-(4-Methoxybenzylidene)pyridin-2-amine (**8**):* Yield 84%, m.p. 158°C; Anal. Calcd. for $C_{13}H_{12}N_2O$; C, 73.56.; H, 5.70; N, 13.20. Found: C, 73.45; H, 5.58; N, 13.08%; IR (KBr) ν_{max}: 1110, 1510, 1575, 1612, 2975, 3035 cm^{-1}; ^1H NMR ($CDCl_3$): 3.63 (3H, s, -OC***H***$_3$), 6.82 (1H, m, Ar***H***), 7.01 (2H, d, *J* 8.8 Hz, Ar***H***), 7.44 (1H, m, Ar***H***), 7.79 (1H, m, Ar***H***), 7.93 (2H, d, *J* 8.8 Hz, Ar***H***), 8.51 (1H, m, Ar***H***), 9.15 (1H, s, N=C***H***).

*N-(4-Dimethylaminobenzylidene)pyridin-2-amine (**9**):* Yield 78%, m.p. 68°C; Anal. Calcd. for $C_{14}H_{15}N_3$; C, 74.64.; H, 6.71; N, 18.65. Found: C, 74.55; H, 6.59; N, 18.55%; IR (KBr) ν_{max}: 1130, 1508, 1575, 1620, 2965, 3070 cm^{-1}; ^1H NMR ($CDCl_3$): 2.91 (6H, s, -N(C***H***$_3$)$_2$), 6.76 (2H, d, *J* 8.8 Hz, Ar***H***), 6.92 (1H, m, Ar***H***), 7.52 (1H, m, Ar***H***), 7.75 (1H, m, Ar***H***), 7.64 (2H, d, *J* 8.8 Hz, Ar***H***), 8.51 (1H, m, Ar***H***), 9.11 (1H, s, N=C***H***).

*2-Methoxy-4-((pyridine-2-ylimino)methyl)phenol (**10**):* Yield 86%, m.p. 138°C; Anal. Calcd. for $C_{13}H_{12}N_2O_2$; C, 68.41; H, 5.30; N, 12.27. Found: C, 68.24; H, 5.17; N, 12.21%; IR (KBr) ν_{max}: 1075, 1502, 1595, 1610, 2960, 3040, 3320 cm^{-1}; ^1H NMR ($CDCl_3$): 3.72 (3H, s, -OC***H***$_3$), 4.72 (1H, brs, O***H***), 6.88 (1H, m, Ar***H***), 7.48–7.55 (3H, m, Ar***H***), 7.73 (1H, m, Ar***H***), 7.09 (1H, m, Ar***H***), 8.51 (1H, m, Ar***H***), 9.15 (1H, s, N=C***H***).

*2-((Pyridine-2-ylimino)methyl)phenol (**11**):* Yield 96%, m.p. 64°C; Anal. Calcd. for $C_{12}H_{10}N_2O$; C, 72.71; H, 5.08; N, 14.13. Found: C, 72.59; H, 4.93; N, 14.01%; IR (KBr) ν_{max}: 1120, 1515, 1580, 1620, 3075, 3290 cm^{-1}; ^1H NMR ($CDCl_3$): 6.93–7.12 (3H, m, Ar***H***), 7.56–7.78 (4H, m, Ar***H***), 8.51 (1H, m, Ar***H***), 9.44 (1H, s, N=C***H***), 13.47 (1H, brs, -O***H***).

*2-Methoxy-6-((pyridine-2-ylimino)methyl)phenol (**12**):* Yield 98%, m.p. 82°C; Anal. Calcd. for $C_{13}H_{12}N_2O_2$; C, 68.41; H, 5.30; N, 12.27. Found: C, 68.23; H, 5.22; N, 12.15%; IR (KBr) ν_{max}: 1110, 1508, 1602, 1625, 2960, 3050, 3282 cm^{-1}; ^1H NMR ($CDCl_3$): 3.68 (3H, s, -OC***H***$_3$), 6.88–7.32 (5H, m, Ar***H***), 7.75 (1H, t, *J* 8 Hz, Ar***H***), 8.51 (1H, m, Ar***H***), 9.46 (1H, s, N=C***H***), 14.02 (1H, brs, -O***H***).

*5-Methoxy-2-((pyridine-2-ylimino)methyl)phenol (**13**):* Yield 97%, m.p. 72°C; Anal. Calcd. for $C_{13}H_{12}N_2O_2$; C, 68.41; H, 5.30; N, 12.27. Found: C, 68.19; H, 5.20; N, 12.21%; IR (KBr) ν_{max}: 1110, 1508, 1605, 1625, 2960, 3050, 3292 cm^{-1}; ^1H NMR ($CDCl_3$): 3.76 (3H, s, -OC***H***$_3$), 6.56–7.34 (4H, m, Ar***H***), 7.69–7.76 (2H, m, Ar***H***), 8.51 (1H, m, Ar***H***), 9.45 (1H, s, N=C***H***), 14.05 (1H, brs, -O***H***).

*2-Ethoxy-6-((pyridine-2-ylimino)methyl)phenol (**14**):* Yield 98%, m.p. 94°C; Anal. Calcd. for $C_{14}H_{14}N_2O_2$; C, 69.41; H, 5.82; N, 11.56. Found: C, 69.27; H, 5.72; N, 11.41%; IR (KBr) ν_{max}: 1110, 1506, 1585, 1618, 2970, 3058, 3299 cm^{-1}; ^1H NMR ($CDCl_3$): 1.51 (3H, t, *J* 6.8 Hz -OCH$_2$C***H***$_3$), 4.13 (2H, t, *J* 6.8 Hz, -OC***H***$_2$CH$_3$), 6.85–7.30 (5H, m, Ar***H***), 7.75 (1H, t, *J* 8 Hz, Ar***H***), 8.51 (1H, m, Ar***H***), 9.45 (1H, s, N=C***H***), 14.03 (1H, brs, -O***H***).

*4-Bromo-2-methoxy-6-((pyridine-2-ylimino)methyl)phenol (**15**):* Yield 98%, m.p. 92°C; Anal. Calcd. for $C_{13}H_{11}BrN_2O_2$; C, 50.84; H, 3.61; N, 9.12. Found: C, 50.69; H, 3.45; N, 9.02%; IR (KBr) ν_{max}: 642, 1112, 1498, 1590, 1615, 2955, 3060, 3302 cm^{-1}; ^1H NMR ($CDCl_3$): 3.66 (3H, s, -OC***H***$_3$), 6.85–7.44 (4H, m, Ar***H***), 7.76 (1H, m, Ar***H***), 8.51 (1H, m, Ar***H***), 9.43 (1H, s, N=C***H***), 14.05 (1H, brs, -O***H***).

Biological assay

Determination of radical scavenging activity using 2,2´-diphenyl-1-picrylhydrazyl (DPPH) radical:

The DPPH radical scavenging ability of all the prepared azomethines was evaluated as previously delineated by Blois with some modifications[55,56]. Briefly, the compounds were dissolved in minimum volume of DMSO and diluted with PBS buffer. Different concentrations (1–100 μM) of the compounds

under investigation were mixed with 0.1 mM methanolic solution of DPPH (SRL, India) and incubated for 30 min in dark at 37°C. Absorbance of the solution was determined with a digital colorimeter (Labtronics) at λ = 517 nm. L-Ascorbic acid and DMSO were employed as the standard positive and negative controls respectively. The following equation was used to find out the percentage of DPPH radical scavenging activity:

$$\% \text{ Scavenging of DPPH} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where $A_{control}$ represents the absorbance of the control reaction (with all reagents besides the compound under investigation), and A_{sample} indicates the absorbance displayed by the test sample. Calculation of concentration of the compound showing 50% radical inhibitory activity (IC_{50}) was based on Linear regression analysis. The experiment was carried out three times and the data presented as the mean of three independent determinations.

Assessment of antibacterial activity:

Bacterial strains:

Bacillus subtilis (Gram-positive) and *Escherichia coli* (Gram-negative) strains from American Type Culture Collection (ATCC) were used to examine the antimicrobial activity. Mueller-Hinton (M-H) agar medium (Hi Media, India) was used to maintain the bacterial strains in slants at 4°C, and sub cultured periodically.

Determination of in vitro antibacterial activity using agar-well diffusion method:

The antibacterial activity of prepared azomethines were carried out *in vitro* against the above two bacterial strains by agar-well diffusion assay[57,58]. Briefly, a number of colonies were picked up from the bacterial stock culture and transferred to sterile nutrient broth (5 mL) and incubated for 18 h at 37°C. Each bacterial culture was suspended in saline solution and adjusted to the final inoculum density of 1×10^7 CFU/mL (by 0.5 McFarland standard) on molten M-H agar plates. Once the agar was solidified, wells of uniform diameter (5 mm) were made with a sterile borer in the inoculated agar plates. 20 µL solution containing different concentrations (0.05–0.5 mg/mL) of prepared bromo derivatives was dispensed separately in each well under aseptic conditions. Ampicillin (Sigma-Aldrich, USA) was the standard drug used as positive controls (0.1 mg/mL) while DMSO was tested as vehicle control in this study. The plates were kept at room temperature for 2 h to permit the diffusion of extracts into the agar and then incubated at 37°C for a further period of 24 h. The diameters of the respective inhibition zones around each well were measured. Each experiment was performed in duplicate to confirm the reproducibi-lity and the best results were recorded.

Conclusion

A number of azomethines were prepared in good to nearly quantitative yields from 2-aminopyridine and various aromatic aldehydes through mechanochemical protocol. The methodology was found to be much superior in comparison to the conventional procedure with respect to yield of the product, reaction time and operational simplicity. Scrutiny of the effect of ring substituents showed that the reaction was almost insensitive to the electronic nature of the substituents. In contrast presence of an ortho hydroxy functionality in the nucleus of aromatic aldehydes led to completion of the reactions in a flash. Some of the prepared azomethines were found to exhibit appreciable radical scavenging and antibacterial activity. Remarkable rate enhancement caused by ortho hydroxy function coupled with promising antimicrobial activity found in azomethines with hydroxylated aromatic nuclei prompt us to undertake such studies on similar systems in future.

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