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# Synthesis and Characterization of Some Isoxazole Derivatives of Vanillin as Antimicrobial Agent

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***Abstract:***
*Some new 5-(p-Methoxyphenyl)-3-[3'-methoxy-4'-(p-methylbenzyloxy)phenyl]-isooxazole were prepared. All the prepared compounds were characterized by their spectral (I.R., N.M.R., Mass) data and screened for their antimicrobial activities.*

***Key words:*** *Chalcone, Isoxazole, Antimicrobial activities*

**1. Introduction**

The chemistry of chalcones has generated interest in scientific studies throughout the world, especially for their biological and industrial applications. Kostanecki and Tambor[1] gave the name chalcone. Different methods are available for the synthesis of chalcones. The most convenient method is the one that involves the Clasien-Schmidt condensation of equimolar quantities of aryl methyl ketones with aryl aldehyde in the presence of alcoholic alkali[2-4] . Chalcone derivatives have been found to possess wide range of therapeutic activities such as Antitumor[5], Antispasmodic[6], Antiulcer[7], Anthelmintics[8] , Bactericidal[9] , Cardiovascular[10], Fungicidal[11] , Germicidal[12], Herbicidal[13] , Insecticidal[14], Antiviral[15].

In 1888 Claisen first suggested the synthesize for isoxazole from the reaction of 1,3 diketone with hydroxyl amine[16] subsequently a solid foundation for the chemistry of isoxazoles was laid down by Claisen and his students. It was shown to possess typical properties of an aromatic system by under certain reaction conditions. Particularly in reducing or basic media it becomes very highly labile. Isoxazole derivatives exhibit various biological activities such as, Antipyretic[17], Anticonvulsant[18] , Anticholestermic[19], Anticancer[20], Anthelmintics[21], Antiinflammatory[22], Adenosine antagonist[23], Fungicidal[24], Herbicidal[25] , Hypoglycemic[26], Musclerelaxant[27], Nematocidal[28] , Insecticidal[29], Antiviral[30], Antimicrobial[31].

The structure of synthesized compounds were assigned based on Elemental analysis, I.R. $^1$H-NMR and Mass spectral data. The antimicrobial activity was assayed by using the cup-plate agar diffusion method [32] by measuring the zone of inhibition in mm. All the compounds were screened *in vitro* for their antimicrobial activities[33] against varieties of bacterial strains such as *Staphylococcus subtilis, Escherichia coli, Proteus vulgaris* and fungi *Aspergillus niger* at 40 µg concentration. Standard drugs like Ampicillin, Amoxicillin, Norfloxacin, Benzyl penicillin and Griseofulvin were used for comparison purpose (Table-2).

**2. Experimental Section**

Melting points were taken in open capillary and are uncorrected. IR spectra ($cm^{-1}$) were recorded on Shimadzu-435-IR Spectrophotometer and $^1$H-NMR spectra on Bruker spectrometer (300MHz) using TMS as an internal standard, chemical shift is in δ ppm.

*2.1. Preparation of 3-Methoxy-4-(p-methylbezyloxy)-benzaldehyde :*

The solution of Vanillin (1.53g, 0.01M) in DMF (7.7ml) was heated at 50-55$^0$C in presence of 4-methylbenzyl chloride (1.75g, 0.01) and $K_2CO_3$ (2.76gm, 0.02) for 5 hrs, after 5 hrs product was precipitated by adding the solution in to cold water. The separated solid was filtered and crystallized from ethanol. Yield 90%, m.p.84$^o$C.

*2.2. 3-Methoxy-4-(p-methylbezyloxy)-benzaldehyde: (A)*

Yield 90%, m.p. 84 $^0$C; IR(KBr) : ν 3032(C-H Str.), 1273 (-$OCH_3$) , 1226 (Ar-O-C) $cm^{-1}$, $^1$H-NMR ($CDCl_3$) : δ 9.83 (s,1H,-CHO) , 5.21 (s,2H,-O-$CH_2$-) 6.96-7.41 (m,7H, Ar-H) 3.94 (s,3H,-$OCH_3$) 2.95 (s,3H,-$CH_3$) . Mass m/z 256 M.F.:$C_{16}H_{16}O_3$. M/z: 112,134,161,213,226,255.

*2.3. Preparation of 1-Aryl-3-[4'-(p-methylbenzyloxy)-3'-methoxyphenyl]-propenones (1b) :*
A mixture of 3-Methoxy-4-(p-methylbenzyloxy)-benzaldehyde (A) (0.01M) and 4-methoxy acetophenone (0.01) was taken in methanol, NaOH (0.002M) was added to the reaction mixture. The reaction mixture was then magnetically stirred for 12 hrs and left overnight. It was poured over crushed ice and neutralized with dilute HCl. It was crystallized from ethanol. Similarly, other chalcones were prepared and its physical data are recorded in Table no.1

*2.4. 1-(p-Methoxyphenyl)-3-[4'-(4-methylbenzyloxy)-3'-methoxyphenyl]-propenones (1b):*
Yield 76%, m.p. 112$^0$C; IR(KBr) : ν 2968, 2841,1456 (Alkane,-$CH_3$), 1255 (-$OCH_3$) , 1255 (Ar-O-C) , 1653 (C=O) ,1591 (C=C) ,C=C Str.1512,C-H i. p.(def.) 1133,C-Ho.o.p.(def.) 817cm$^{-1}$; $^1$H-NMR ($CDCl_3$) : δ 2.26 (s,3H,-$CH_3$) 3.84 & 3.86, (s,6H,-$OCH_3$) ,7.33 & 7.67 (d,2H,-CH=CH-), 5.09 (s,2H,-O-$CH_2$-) ,6.80-7.96 (m,11H, Ar-H) . Mass m/z 388. M.F.: $C_{25}H_{24}O_4$. m/z: 43,77,92,105,135,176,284,388.

*2.5. Preparation of 3-(p-Methoxyphenyl-5-[3'-methoxy-4'-(p-methylbenzyloxy)phenyl]-isoxazole (2b) :*
A mixture of Hydroxylamine hydrochloride (0.01M), 1-Aryl-3-[4'-(p-methylbenzyloxy )-3'-methoxyphenyl]-propenones (1a-l) (0.01M) and $CH_3COONa$ (0.01M) in ethanol was refluxed with stirring for about 6-8 hrs until the reaction was complete, which was monitored as formation of precipitate of isoxazole took place. Similarly, other isoxazoles were prepared and its physical data are recorded in Table no.1

*2.6. 3-(p-Methoxyphenyl)-5-[3'-methoxy-4'-(p-methylbenzyloxy) phenyl]-isoxazole :*
Yield 68%, m.p. 121 $^0$C; IR(KBr) : ν 2938, 3049,1466 (Alkane,-$CH_3$), 1252 (-$OCH_3$) ; 1030 (Ar-O-C) , 1605 (C=N) , 833(N-O), 1595, 1112, 833 (Aromatic) ; $^1$H-NMR ($CDCl_3$) : δ 2.35 (s,3H,-$CH_3$) , 3.86, (s,6H,-$OCH_3$) ,5.09 (s,2H,-O-$CH_2$-) ,6.80-7.96 (m,12H, Ar-H). Mass m/z 401 . M.F.:$C_{25}H_{23}O_4N$. m/z : 41,51,77,92,105,135,147,210,296,388,401.

**3. Results and Discussion**
The synthesis of 1-Aryl-3-[4'-(p-methylbenzyloxy)-3'-methoxy-phenyl]-propenones (1a-l) and 3-Aryl-5-[3'-methoxy-4'-(p-methylbenzyloxy)phenyl]-isooxazole (2a-l) was carried out in two steps, first by the condensation of 3-Methoxy-4-(p-methylbenzyloxy)-benzaldehyde (A) with different aromatic acetophenones by Claisen-Schmidt condensation in presence of basic catalyst to give chalcone derivatives (1a-l), which in next step were refluxed with hydroxyl amine hydrochloride and sodium acetate to yield isoxazole derivatives ( 2a-l). (scheme-1).
The formulas of the selected compounds were confirmed by the elemental analysis and their structures were determined by IR , $^1$H-NMR and mass spectral data.

**Characterization data of the compounds (1a-l) and (2a-l)**

| Compd. no. | R | Molecular Formula | Mole.Wt. | M.P. ($^0$C) | Nitrogen % | |
|---|---|---|---|---|---|---|
| | | | | | Calcd. | Found |
| 1a | -$C_6H_5$ | $C_{24}H_{22}O_3$ | 358.0 | 122 | - | - |
| 1b | -4-$NH_2$-$C_6H_4$ | $C_{24}H_{23}NO_3$ | 373.0 | 170 | 3.75 | 3.71 |
| 1c | -4-Br-$C_6H_4$ | $C_{24}H_{21}O_3Br$ | 437.0 | 112 | - | - |
| 1d | -4-Cl-$C_6H_4$ | $C_{24}H_{21}ClO_3$ | 392.5 | 108 | - | - |
| 1e | -2,4-$(Cl)_2$-$C_6H_3$ | $C_{24}H_{20}Cl_2O_3$ | 427.0 | 140 | - | - |
| 1f | -2-OH-$C_6H_4$ | $C_{24}H_{22}O_4$ | 374.0 | 73 | - | - |
| 1g | -3-OH-$C_6H_4$ | $C_{24}H_{22}O_4$ | 374.0 | 78 | - | - |
| 1h | -4-OH-$C_6H_4$ | $C_{24}H_{22}O_4$ | 374.0 | 72 | - | - |
| 1i | -4-$OCH_3$-$C_6H_4$ | $C_{25}H_{24}O_4$ | 388.0 | 112 | - | - |
| 1j | -4-$CH_3$-$C_6H_4$ | $C_{25}H_{24}O_3$ | 372.0 | 134 | - | - |
| 1k | -3- $NO_2$-$C_6H_4$ | $C_{24}H_{21}O_5N$ | 403.0 | 92 | 3.47 | 3.46 |
| 1l | -4- $NO_2$-$C_6H_4$ | $C_{24}H_{21}O_5N$ | 403.0 | 148 | 3.47 | 3.45 |
| 2a | -$C_6H_5$ | $C_{24}H_{21}03N$ | 371 | 102 | 3.77 | 3.73 |
| 2b | -4-$NH_2$-$C_6H_4$ | $C_{24}H_{22}O_3N_2$ | 386 | 145 | 7.25 | 7.22 |
| 2c | -4-Br-$C_6H_4$ | $C_{24}H_{20}O_3NBr$ | 450 | 125 | 3.11 | 3.10 |
| 2d | -4-Cl-$C_6H_4$ | $C_{24}H_{20}O_3NCl$ | 405 | 85 | 3.45 | 3.40 |
| 2e | -2,4-$(Cl)_2$-$C_6H_3$ | $C_{24}H_{19}O_3NCl_2$ | 440 | 92 | 3.18 | 3.16 |
| 2f | -2-OH-$C_6H_4$ | $C_{24}H_{21}0_4N$ | 387 | 90 | 3.61 | 3.60 |
| 2g | -3-OH-$C_6H_4$ | $C_{24}H_{21}0_4N$ | 387 | 116 | 3.61 | 3.59 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 2h | -4-OH-$C_6H_4$ | $C_{24}H_{21}0_4N$ | 387 | 101 | 3.61 | 3.58 |
| 2i | -4-$OCH_3$-$C_6H_4$ | $C_{25}H_{23}NO_4$ | 401 | 121 | 3.49 | 3.48 |
| 2j | -4-$CH_3$-$C_6H_4$ | $C_{25}H_{23}NO_3$ | 385 | 114 | 3.63 | 3.61 |
| 2k | -3-$NO_2$-$C_6H_4$ | $C_{24}H_{20}N_2O_5$ | 416 | 78 | 6.73 | 6.70 |
| 2l | -4-$NO_2$-$C_6H_4$ | $C_{24}H_{20}N_2O_5$ | 416 | 90 | 6.73 | 6.71 |

*Table 1*

*3.1. Antibacterial Activity*

It has been observed from the microbiological data that all compounds (1a-l) and (2a-l) were found to be mild to moderately active against Gram positive and Gram negative bacterial strains. How ever the maximum activity was observed in compounds (1c), (1h), (2a), (2l) against *S. aureus*. The significant activity was observed in compounds (1h), (1j), (2j), (2l) against *B. subtilis*. The maximum activity was displayed by the compounds (1e), (1i), (2b), (2g) against *E. coli*. The compounds (1b), (1i), (2h), and (2i) were comparatively more effective against *P.vulgaris*.

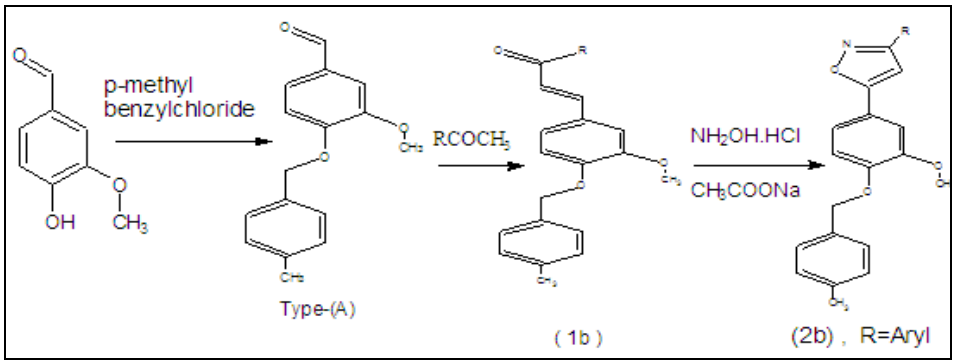
*3.2. Antifungal Activity*

The antifungal data revealed that compounds were least toxic to the fungal strain. However mild activity was shown by the compounds (1e), (1h), (1l), (2b), (2e), (2f) against *A. niger***.**

The antibacterial activity was compared with standard drug viz. Ampicillin, Amoxicillin, Norfloxacin, Benzyl penicillin and antifungal activity was compared with standard drug Griseofulvin.

| **Compd no.** | **Antibacterial activity (zone of inhibition in mm)** | | | | **Antifungal Activity** |
|---|---|---|---|---|---|
| | **S. aureus** | **B. subtilis** | **E. coli** | **P. vulgaris** | **A. niger** |
| 1a | 10 | 17 | 17 | 15 | 16 |
| 1b | 18 | 14 | 15 | 18 | 15 |
| 1c | 20 | 15 | 15 | 16 | 14 |
| 1d | 16 | 17 | 18 | 13 | 13 |
| 1e | 14 | 10 | 19 | 18 | 20 |
| 1f | 15 | 12 | 13 | 17 | 15 |
| 1g | 18 | 16 | 16 | 15 | 17 |
| 1h | 19 | 18 | 15 | 16 | 18 |
| 1i | 12 | 16 | 19 | 19 | 18 |
| 1j | 13 | 19 | 16 | 15 | 16 |
| 1k | 18 | 15 | 15 | 14 | 17 |
| 1l | 12 | 17 | 17 | 11 | 19 |
| 2a | 21 | 21 | 22 | 16 | 13 |
| 2b | 17 | 20 | 24 | 22 | 22 |
| 2c | 15 | 15 | 21 | 23 | 20 |
| 2d | 20 | 13 | 19 | 21 | 18 |
| 2e | 15 | 23 | 20 | 19 | 22 |
| 2f | 16 | 17 | 18 | 18 | 23 |
| 2g | 19 | 14 | 23 | 22 | 19 |
| 2h | 15 | 18 | 16 | 24 | 17 |
| 2i | 12 | 15 | 14 | 23 | 20 |
| 2j | 18 | 22 | 16 | 17 | 21 |
| 2k | 20 | 18 | 19 | 20 | 16 |
| 2l | 21 | 22 | 20 | 19 | 17 |
| Ampicillin | 22 | 20 | 21 | 24 | 0 |
| Amoxicillin | 20 | 23 | 22 | 21 | 0 |
| Norfloxacin | 19 | 20 | 23 | 22 | 0 |
| Benzyl penicillin | 21 | 21 | 19 | 18 | 0 |
| Griseofulvin | 0 | 0 | 0 | 0 | 25 |

*Table 2*




*Scheme 1*

**4. Conclusion**

The present study leads to a convenient synthetic method for the synthesis of new compounds. Which showed significant antibacterial and antifungal activity. Further investigation with appropriate structural modification of the above compounds may result in therapeutically useful products.

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