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Zinc Oxide (ZnO): An Efficient Catalyst for the Synthesis of 4-arylmethylidene-2-phenyl 5(4*H*)-oxazolones Having Antimicrobial Activity

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Abstract: A straightforward and a general method has been developed for the synthesis of some important functionalized 4-arylmethylidene-2-phenyl-5(4*H*)-oxazolone derivatives by combining araldehydes and hippuric acid in the presence of catalytic amount of zinc oxide at room temperature in ethyl alcohol is reported. The method was very easy, rapid and products obtained were in excellent yields. The compounds were purified, characterized and were subjected for *in vitro* antibacterial activity (determination of *Minimum Inhibitory Concentration* (MIC) by micro titration method) using two standard drugs Streptomycin and Ampicillin against *Bacillus subtilis* and *Escherichia coli*. Five of the synthesized compounds showed remarkable antibacterial activity when compared to that of standards.

Key words: 4-Arylmethylidene-2-phenyl-5(4*H*)-oxazolones, araldehydes, hippuric acid, Zinc oxide (ZnO), Room temperature, antibacterial activity, Minimum Inhibitory Concentration

INTRODUCTION

4-arylmethylidene -2-phenyl-5(4*H*)-oxazolones are widely used in the synthesis of many natural products (Cannella *et al*., 1996). These molecules have biological activity and find applications in pharmacological studies (Gelmi *et al*., 1997). Some oxazolones are highly versatile intermediates; used for the synthesis of several organic molecules including aminoacids (Bautista *et al*., 1992), peptides (Cavalier and Verducci, 1995; Gottwald and Seebach, 1999; Meiwes *et al*., 1997; Seebach *et al*., 1997; Cativiela *et al*., 1997; Bun˜nel *et al*., 1995; Donati *et al*., 1996), antimicrobial and antitumor compounds (Martinez *et al*., 1964), heterocyclic precursors (Avenoza *et al*., 2002; Croce *et al*., 1994; Cannella *et al*., 1996), immunomodulators (Mesaik *et al*., 2004), biosensors or photosensitive composition devices for proteins (Penalba *et al*., 2000; Kojima *et al*., 1998). Due to pharmacological and other applications (Cativiela *et al*., 1996), synthesis of 4-arylmethylidene-2-phenyl-5(4*H*)-oxazolones has gained acceptance and popularity. The preparation of oxazolone derivatives is achieved by the condensation of araldehyde, hippuric acid and acetic anhydride in the presence of various reagents and catalysts such as $Pb(OAc)_2$ (Cativiela *et al*., 1999; Cativiela and Melendez, 1978), Al_2O_3-H_3BO_3 (Ringold *et al*., 1956), Bi(III) salts (Khodaei *et al*., 2003), $ZnCl_2$ (Rao and Venkataraman, 1994) and Montmorillonite K-10 (Karade *et al*., 2005). Under microwave irradiation using $Ca(OAc)_2$ (Paul *et al*., 2004), $Pd(OAc)_2$ (Hamidian and Tikari, 2006) and KF/NaOAc (Bautista *et al*., 2002). Many of the existing methods involve expensive reagents, strongly acidic conditions, require longer reaction duration, high temperatures, incompatible with other functional groups, involve cumbersome product isolation and give unsatisfactory yields.

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Recently Paul *et al*. (2004) and Hamidian and Tikari (2006), have reported the synthesis of 4-arylmethylidene-2-aryl-5(*4H*)-oxazolones from arylaldehydes, hippuric acid and acetic anhydride in the presence of catalytic amounts of calcium acetate or palladium acetate respectively under microwave irradiation in good yields, where chemistry community fails to carryout reactions on a large-scale.

MATERIALS AND METHODS

Melting points were determined on a Büchi melting point apparatus. IR spectra were recorded on Nicolet 400D FT-IR spectrophotometer, ^{1}H-NMR, ^{13}C-NMR were recorded on 200 MHZ Bruker spectrometer, GC-MS using Shimadzu GC-MS QP 5050A spectrometer and elemental analysis was performed on Themo Finnigan FLASH EA 1112 CHNS analyzer. Araldehydes, hippuric acid and ZnO were all AR grade and were used without purification.

Minimum Inhibitory Concentration (MIC) of the synthesized compounds was determined using micro-titration method, where *Luria broth medium* was used as medium for MIC determination.

Antibacterial Activity

Determination of Minimum Inhibitory Concentration (MIC)

The determination of minimum inhibitory concentration (Elisabeth Lowdin *et al*., 1993; Kotretsou *et al*., 1995) was done with the *Escherichia coli* and *Bacillus subtilis* two isolates were inoculated into *Luria broth medium* which contains 1% tryptone, 0.5% yeast extract and 0.5% sodium chloride; the pH of the medium was adjusted to 7.2 with sterile phosphate buffered saline and incubated at 37°C for 24 h. The optical density of the bacteria from mid log phase of growth was measured at 540 nm and diluted in fresh medium so as to get an optical density of 0.004 (corresponding to 5×10^{5} colony forming units/mL. To each well of the ELISA plate (Corning, USA), 200 µL of diluted bacterial suspension was added and graded concentrations (0.2-500 µg/50 µL) of the synthesized compounds and two standard antibiotics (*Streptomycin* and *Ampicillin*) in dimethyl sulfoxide were added and incubated at 37°C for 24 h. At the end of incubation, the effect of the drugs on the growth of organisms was monitored by measuring the optical density at 540 nm using ELISA reader (Multiscan MS, Labsystems, Helsinki, Finland). The MIC was defined as the lowest concentration of the antibiotic or test sample allowing no visible growth. Determination of MICs was performed in triplicate and the results are tabulated in Table 2.

General Procedure for 4-(4'-flurobenylidene)-2-phenyloxazol-5(4*H*)-one

A mixture of *p*-fluorobenzaldehyde (1.24 g, 10 mmole), hippuric acid (1.79 g, 10 mmole), acetic anhydride (30 mmol), catalytic amount of ZnO (dry powder 0.49 mg, 6 mmole) and 10 mL ethyl alcohol were serried at room temperature (25°C), the syrupy reaction mixture solidified for a certain period of time (Table 2) as required to complete the reaction at room temperature (25°C). The reaction mixture was added to cold ethyl alcohol (30 mL) for extraction, the solid obtained after solvent evaporation was washed with hot water (2×10 mL), dried and recrystallized (C_2H_5OH:H_2O) to afford pure yellow colored crystals of title compound (3m) (2.61 g, 98%).

Spectral Data

4-(4'-Flurobenylidene)-2-phenyloxazol-5(4*H*)-one (3m): IR (Kbr) cm^{-1} 1794, 1658, 1595, 1560, 1496, 1172, 834, 690; ^{1}H-NMR ($CDCl_3$+DMSO) δ 7.15 (s, 1H), 7.40 (d, 2H, J = 8.3 Hz), 7.52-7.64 (m, 3H) 8.17-8.25 (m, 4*H*); ^{13}C-NMR ($CDCl_3$+DMSO) δ 124.73(C), 128.23(CH), 129.14(CH), 129.17(CH), 129.57(CH), 132.11(C), 132.69(CH), 133.86(C), 133.94(C), 164.79(C), 166.80(C); *Elemental Anal.* Calculated for $C_{16}H_{10}N$: C, 71.91; H, 3.76; N, 5.23; Found: C, 71.82; H, 3.70; N, 5.18; MS: m/z = 267 (M^+).

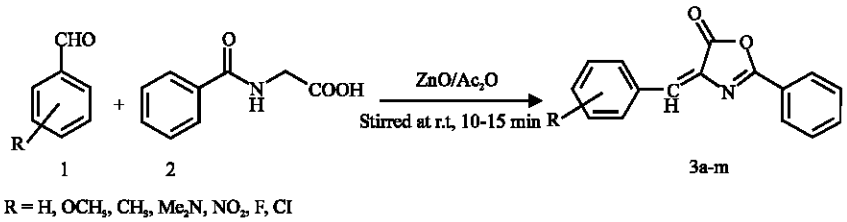
RESULTS AND DISCUSSION

In continuation of our investigation on the use of heterogeneous catalysts for the preparation of medicinally important molecules under environmentally safe conditions (Pasha and Jayashankara, 2006a-f; Pasha *et al*., 2006), we have found that, ZnO, which is an inexpensive, readily available and common laboratory chemical, can efficiently catalyze this reaction. We have been able to use ZnO as a catalyst for the synthesis of 4-arylmethylidene-2-phenyl-5(4*H*)-oxazolones (3a-m) from araldehydes (1), hippuric acid (2) and acetic anhydride in ethyl alcohol at room temperature in about 20 min as shown in Scheme 1.

To demonstrate the protocol, we selected *p*-anisaldehyde as the model substrate and treated with hippuric acid, acetic anhydride and catalytic amount of ZnO in ethyl alcohol at room temperature for 10 min to obtain the desired 4-arylmethylidene-2-phenyl-5(4*H*)-oxazolone (95%, entry 6, Table 1). Comparison of existing catalysts and different methodologies is also shown in Table 1.

The method was extended to obtain the remaining compounds and check for their antibacterial activity to determine *minimum inhibitory concentration* using two standard drugs *Streptomycin* and *Ampicillin*; the results are tabulated in Table 2.

When compared to other reported methods, we have found that ZnO is an efficient catalyst for the preparation of 4-arylmethylidine-2-aryl-5(4*H*)-oxazolones (3 a-m) in high yields, under mild reaction condition, in a short duration of time. Several interesting features of the preparation of 4-arylmethylidene-2-phenyl-5(4*H*)-oxazolones are apparent from Table 2. More importantly, the substituents such as OCH_3, Cl, F and NO_2 are unaffected under the reaction condition. Thus, the results summarized in the Table 2 clearly indicate the scope of the reaction with respect to various substituted aromatic aldehydes.



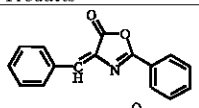
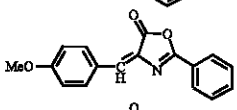
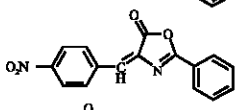
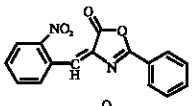
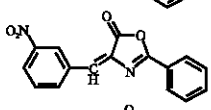
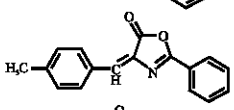
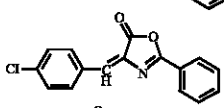
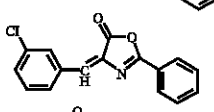
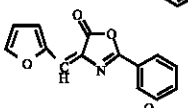
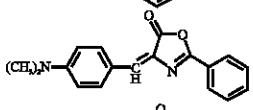
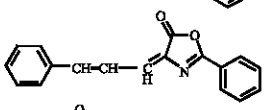
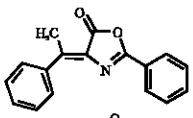
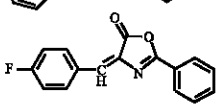
Scheme 1

Table 1: Comparison of the reported methods for the condensation of *p*-anisaldehyde with hippuric acid in the presence of different catalysts

Entry	Catalyst	Time	Temp. (°C)	Yield (%)[b]
1	Mont.K-10	6 h	Reflux	89
2	Bi(III) salts	1 h	Reflux	75-88
3	$Ca(OAc)_2$	3 min	MW	99
4	$Pd(OAc)_2$	3 min	MW	98
5	KF/NaOAc	15 min	MW	78
6	ZnO (6 mmole)[a]	10 min	Room temp.	95

[a]Present method: p-anisaldehyde (10 mmole), hippuric acid (10 mmole) and acetic anhydride (30 mmole) were stirred together to afford 3b. [b]isolated yields

Table 2: Conversion of substituted arylaldehyde to their corresponding 4-arylmethylidene-2-phenyl-5(4*H*)-oxazolones(3a-m) in the presence ZnO

Entry	Products[a]	Yield[b] (%)	Time (min)	Melting point (°C) Found	Melting point (°C) Reported	Antibacterial Activity (MIC) *Bacillus subtilis* MIC (μg/50 μL)	Antibacterial Activity (MIC) *Escherichia coli* MIC (μg/50 μL)
3a		90	10	170-71	170	230.6	233.6
3b		95	10	165	165	259.2	243.2
3c		92	10	240-41	241	268.8	256.0
3d		92	10	165-66	166	281.6	307.2
3e		92	10	195	195-96	272.0	294.4
3f		94	15	145	145-46	180.8	167.0
3g		95	10	204-05	203-04	179.2	160.0
3h		95	10	155	156	185.6	182.4
3i		92	15	170	171	204.6	230.8
3j		90	15	210-12	213	192.0	243.6
3k		92	15	130-32	132	230.4	180.0
3l		95	15	105-06	106-107	205.2	192.4
3m		98	10	169-170	-	166.4	160.4
Streptomycin						128.8	134.4
Ampicillin						131.2	132.8

[a]Products 3a-3l are known (ref, 11,13,14 and 15) and compound 3m is new; all the products were characterized by IR, ^{1}H-NMR, GC-MS and CHN analysis and compared with the authentic samples

CONCLUSIONS

In conclusion, we demonstrated an efficient synthesis of 4-arylmethylidene-2-aryl-5(4*H*)-oxazolones using a catalytic amount of ZnO under room temperature. Out of the thirteen compounds synthesized four compounds 3f, 3g, 3h and 3m showed significant MIC at 180.8, 179.2, 185.6 and 166.4 μg/50 μL, respectively against *Bacillus subtilis*. Compound 3j showed medium MIC activity at 192.0 μg/50 μL against same organism. Compounds 3f, 3g, 3h, 3k and 3m also exhibited MIC at 167.0, 160.0, 182.4, 180.0 and 160.4 μg/50 μL, respectively against *Escherichia coli*. Compound 3l showed medium MIC at 192.4 μg/50 μL against *Escherichia coli*. One of the methyl derivatives i.e., 4-(*p*-methyl benzylidene)-2-phenyl-(4*H*)-oxazol-5-one also exhibited significant activity at 180.8 and 167.0 μg/50 μL against *Bacillus subtilis* and *Escherichia coli*. The above observations revealed that halogen derivatives of substituted oxazolones seem to have significant antibacterial activity against two strains of the experimental organisms.

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