SYNTHESIS OF NOVEL METHYLENE-BIS-ISOXAZOLES AS POTENTIAL FUNGICIDAL AGENTS

Ch. Sanjeeva Reddy* and A. Nagaraj
Department of Chemistry, Kakatiya University, Warangal – 506 009, India.
e-mail: chsrkuc@yahoo.co.in

Abstract : A series of novel methylene-bis-chalcones **3** was prepared by the Claisen-Schmidt condensation of 5,5'-methylene-bis-salicylaldehye **2** with various acetophenones, subsequent condensation of compound **3** with hydroxylamine hydrochloride gave the corresponding novel methylene-bis-isoxazoles **4** in good yields. Characterization of the new compounds has been done by means of IR, ^{1}H NMR, MS and elemental analyses. All the compounds have also been screened for their antifungal activity and some of them showed quite comparable activity with the standard antibiotics.

Key words : Methylene-bis-chalcones, methylene-bis-isoxazoles, antifungal activity.

Introduction

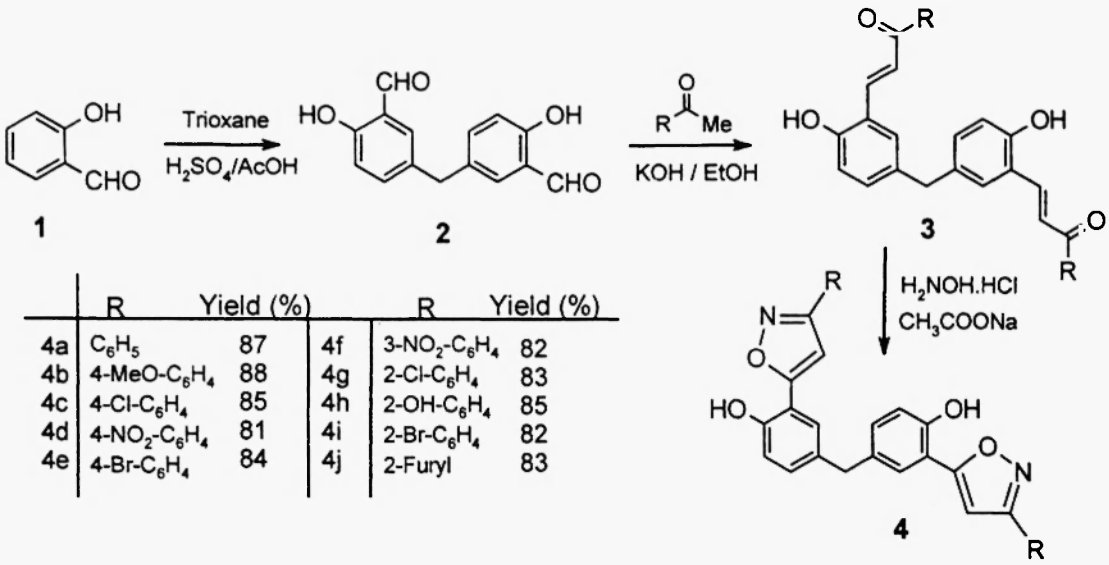
Heterocyclics are abundant in nature and are of great significance to life because of their structural subunits exists in many natural products such as vitamins, hormones, antibiotics, alkaloids as well as pharmaceuticals, herbicidal, dyes and many more compounds[1], hence have attracted considerable attention in the design of biologically active molecules[2] and advanced organic materials[3]. A practical method for the synthesis of such compounds is of great interest in synthetic organic chemistry. Among the heterocycles isoxazoles are a class of compounds with biological and pesticidal activities[4]. The most general and widely employed synthetic route to isoxazoles involves reaction of chalcones with hydroxylamine hydrochloride[5] or 1,3-dipolar cycloaddition of nitrile oxides to activated olefins[6] or condensation of open chain α-hydroxymethylene ketones with hydroxylamine[7] or from 3,5-diarylcyclo-hexenone and hydroxylamine[8].

In view of these observations and in continuation of our work on biologically active heterocycles[9] and their increasing importance in pharmaceutical and biological field, it was considered worthwhile to synthesize certain new chemical entities incorporating two active isoxazole nuclei in a single molecular frame work, would furnish compounds of enhanced biological properties. Hence, the synthesis and fungicidal activity of isoxazoles was undertaken. In this regard, methylene-bis-chalcones **3** would be suited for preparing methylene-bis-isoxazoles **4**.

Results and discussion

In this article, we describe the synthesis of novel methylene-bis-isoxazoles **4** from methylene-bis-chalcones **3** and hydroxylamine hydrochloride. For the synthesis of target compounds the 5,5'-methylene-bis-salicylaldehyde **2** was prepared by the reaction of salicylaldehyde **1** with trioxane in the presence of a mixture of acetic acid and concentrated sulfuric acid[10], followed by Claisen-Schmidt condensation of compound **2** with substituted acetophenones in presence of 60% aqueous KOH at room temperature[9d] yields methylene-bis-chalcones **3**. The reaction times as well as the yields vary depending on the corresponding reagents. The crude product contaminated by some starting materials was purified by extracting with ether. The

methylene-bis-chalcones **3** were reacted with hydroxylamine hydrochloride in the presence of sodium acetate in ethanol at reflux temperature for 4 h to get methylene-bis-iosxazoles **4** in good to excellent yields (Scheme 1). The structures of the synthesized compounds were confirmed by IR, ^{1}H NMR, MS and elemental analyses and further, the compounds were screened for their antifungal activity.



	R	Yield (%)		R	Yield (%)
4a	C_6H_5	87	4f	3-NO_2-C_6H_4	82
4b	4-MeO-C_6H_4	88	4g	2-Cl-C_6H_4	83
4c	4-Cl-C_6H_4	85	4h	2-OH-C_6H_4	85
4d	4-NO_2-C_6H_4	81	4i	2-Br-C_6H_4	82
4e	4-Br-C_6H_4	84	4j	2-Furyl	83

Scheme-1

Antifungal activity

The compounds 4(a-j) were screened for their antifungal activity (Table 1) against C. albicans, A. niger and E. vitae at concentrations of 160, 320, 480 and 640 μg/mL, using Agar plate technique[11]. Fluconazole, a standard known antibiotic was also tested under the similar conditions for comparison. From the results it is found that, most of the compounds 4(a-j) inhibited significantly the mycelial growth of three test fungi at 640 μg/mL concentration however, their activity decreased considerably at lower concentrations (320 and 160 μg/mL). The compounds 4b, 4e and 4i have similar activities, quite comparable with the standard antibiotic fluconazole, at 160 and 640 μg/mL concentration and showed ~80% and ~90% growth inhibition respectively against all the test fungi.

The compounds **4c** and **4j** were highly active against *C. albicans* and compound **4g** was highly active against *A. niger* and *E. vitae*. The other compounds showed moderate to good activity. There was a significant alteration in the antifungal activity with the change in the relative position of the substituents on the benzene ring, for example compounds **4b**, **4c** and **4e** bearing –OMe or –Cl or –Br group were more active than **4d**, **4f** and **4h** with $-NO_2$ or –OH group. The results of the antifungal activity are presented in Table 1.

In conclusion, we have described the synthesis of novel methylene-bis-isoxazoles **4** in good to excellent yields by the reaction of methylene-bis-chalcones **3** and hydroxylamine hydrochloride. Some of these compounds exhibit excellent antifungal activity, comparable with the standard antibiotics.

Table-1: Antifungal activity of compounds **4(a-j)**

Compound	Spore germination inhibition (in %) at different conc. (in µg/mL) against											
	C. albicans				*A. niger*				*E. vitae*			
	160	**320**	**480**	**640**	**160**	**320**	**480**	**640**	**160**	**320**	**480**	**640**
4a	34.7	37.2	39.4	40.5	32.1	36.7	40.4	41.3	39.9	44.6	45.9	51.0
4b	84.9	85.7	87.2	91.1	77.3	86.1	89.1	90.7	79.1	86.1	88.8	90.1
4c	77.4	79.3	81.0	83.4	56.3	66.9	79.4	84.3	60.1	66.7	70.3	73.4
4d	22.4	27.3	30.2	37.1	36.7	40.3	49.4	50.1	21.7	29.3	34.7	39.3
4e	76.2	77.0	82.0	83.2	81.9	83.4	86.1	87.4	70.3	80.7	81.0	87.3
4f	52.7	55.2	59.4	63.0	44.1	55.0	58.7	60.0	50.1	51.9	55.3	59.6
4g	72.1	74.3	79.2	80.1	79.3	79.9	81.7	86.3	70.6	80.1	82.2	87.3
4h	45.1	47.2	52.1	57.0	44.7	46.7	49.0	53.2	40.4	50.9	54.3	60.5
4i	82.0	85.9	87.3	91.2	80.9	82.1	86.2	88.1	76.7	80.6	81.3	87.4
4j	67.2	73.1	81.2	85.1	60.6	70.7	80.0	86.6	60.5	69.7	70.4	79.7
Fluconazole	84.0	87.0	95.0	98.0	86.0	89.9	93.1	96.2	80.3	84.7	90.2	95.6

Experimental

Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. IR spectra were obtained on a Perkin-Elmer BX series 5000 FTIR spectrometer, using KBr pellet. ^{1}H NMR spectra were obtained with a Varian Gemini 200 MHz spectrometer and the chemical shifts were reported as parts per million (δ ppm) down field using TMS as an internal standard. Mass spectra were obtained on a VG Micromass 7070H spectrometer operating at 70 eV. Elemental analyses were performed on a Perkin-Elmer 240 CHN elemental analyzer. All solvents and chemicals were purchased from Sigma-Aldrich chemical company and used without further purification.

General procedure for the synthesis of methylene-bis-chalcones 3(a-j). A solution of **2** (2.56 g, 0.01 mol) and the corresponding acetophenone (0.02 mol) in 20 mL of ethanol was treated with 20 mL of 60% aq. KOH solution at 5-10 °C. The reaction mixture was stirred at room temperature for 4 h. It was then diluted with water (50 mL) and extracted with diethyl ether (3 x 20 mL). The aqueous solution was acidified with dilute HCl. The solid obtained was filtered, washed thoroughly with water and purified by crystallization from benzene: MeOH (3:2) to afford pure compound **3**.

General procedure for the synthesis of methylene-bis-isoxazoles 4(a-j). To a mixture of hydroxylamine hydrochloride (0.03 mol) in 10 mL of ethanol and sodium acetate (0.06 mol) in 10 mL of hot acetic acid, was added a solution of **3** (0.01 mol) in 10 mL of ethanol. The reaction mixture was refluxed for 3 h. It was then diluted with water (100 mL) and neutralized with dilute NaOH solution. The product separated after acidification with HCl, was extracted with ether. The solvent was evaporated and the solid obtained was purified by crystallization from ethanol to afford pure product**4**.

4-[4-Hydroxy-3-(3-phenyl-5-isoxazolyl)benzyl]-2-(3-phenyl-5-isoxazolyl)phenol (4a). Yield 87%; mp 124-126 °C; IR (KBr): 3340, 3032, 2957, 1595, 1470, 1030 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.98 (s, 2H, CH_2), 4.52 (s, 2H, OH), 6.19 (s, 2H, Ar-H), 6.72 (s, 2H, Ar-H), 7.10 (d, J = 9.1 Hz, 2H, Ar-H), 7.36-7.75 (m, 12H, Ar-H); MS: m/z 486 (M^+). Anal. calcd for $C_{31}H_{22}N_2O_4$: C, 76.53; H, 4.56; N, 5.76. Found: C, 77.01; H, 4.47; N, 5.67.

4-{4-Hydroxy-3-[3-(4-methoxyphenyl)-5-isoxazolyl]benzyl}-2-[3-(4-methoxyphenyl)-5-isoxazolyl]phe- nol (4b). Yield 88%; mp 176-178 °C; IR (KBr): 3340, 3037, 1595, 1477, 1070, 1035 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.84 (s, 6H, OMe), 3.98 (s, 2H, CH_2), 4.53 (s, 2H, OH), 6.19 (s, 2H, Ar-H), 6.72 (s, 2H, Ar-H), 7.10 (m, 6H, Ar-H), 7.45 (d, J = 9.1 Hz, 2H, Ar-H), 7.70 (d, J = 8.4 Hz, 4H, Ar-H); MS: m/z 546 (M^+). Anal. calcd for $C_{33}H_{26}N_2O_6$: C, 72.52; H, 4.79; N, 5.13. Found: C, 70.17; H, 4.68; N, 5.02.

2-[3-(4-Chlorophenyl)-5-isoxazolyl]-4-{3-[3-(4-chlorophenyl)-5-isoxazolyl]-4-hydroxybenzyl}phen- ol (4c). Yield 85%; mp 149-151 °C; IR (KBr): 3342, 3037, 1594, 1475, 1030, 674 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.98 (s, 2H, CH_2), 4.54 (s, 2H, OH), 6.19 (s, 2H, Ar-H), 6.72 (s, 2H, Ar-H), 7.06 (d, J = 9.1 Hz, 2H, Ar-H), 7.18 (d, J = 8.4 Hz, 4H, Ar-H), 7.39 (d, J = 9.1 Hz, 2H, Ar-H), 7.80 (d, J = 8.4 Hz, 4H, Ar-H); MS: m/z 555 (M^+). Anal. calcd for $C_{31}H_{20}Cl_2N_2O_4$: C, 67.04; H, 3.63; N, 5.04. Found: C, 68.03; H, 3.52; N, 4.96.

4-{4-Hydroxy-3-[3-(4-nitrophenyl)-5-isoxazolyl]benzyl}-2-[3-(4-nitrophenyl)-5-isoxazolyl]phenol (4d). Yield 81%; mp 203-205 °C; IR (KBr): 3354, 3042, 1597, 1470, 1360, 1030 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.98 (s, 2H, CH_2), 4.53 (s, 2H, OH), 6.19 (s, 2H, Ar-H), 6.72 (s, 2H, Ar-H), 7.10 (d, J = 9.1 Hz, 2H, Ar-H), 7.37 (d, J = 9.1 Hz, 2H, Ar-H), 8.00 (d, J = 8.4 Hz, 4H, Ar-H), 8.30 (d, J = 8.4 Hz, 4H, Ar-H); MS: m/z 576 (M^+). Anal. calcd for $C_{31}H_{20}N_4O_8$: C, 64.58; H, 3.50; N, 9.72. Found: C, 64.07; H, 3.61; N, 9.70.

2-[3-(4-Bromophenyl)-5-isoxazolyl]-4-{3-[3-(4-bromophenyl)-5-isoxazolyl]-4-hydroxybenzyl}phen- ol (4e). Yield 84%; mp 182-184 °C; IR (KBr): 3065, 1597, 1472, 1030, 580 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.99 (s, 2H, CH_2), 4.53 (s, 2H, OH), 6.19 (s, 2H, Ar-H), 6.57 (s, 2H, Ar-H), 7.10 (d, J = 9.1 Hz, 2H, Ar-H), 7.40 (d, J = 9.1 Hz, 2H, Ar-H), 7.71 (m, 8H, Ar-H); MS: m/z 644 (M^+). Anal. calcd for $C_{31}H_{20}Br_2N_2O_4$: C, 57.79; H, 3.13; N, 4.35. Found: C, 56.80; H, 3.17; N, 4.26.

4-{4-Hydroxy-3-[3-(3-nitrophenyl)-5-isoxazolyl]benzyl}-2-[3-(3-nitrophenyl)-5-isoxazolyl]phenol (4f). Yield 82%; mp 196-198 °C; IR (KBr): 3350, 3064, 2957, 1595, 1470, 1371, 1030 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.98 (s, 2H, CH_2), 4.55 (s, 2H, OH), 6.20 (s, 2H, Ar-H), 6.72 (s, 2H, Ar-H), 7.11 (d, J = 9.1 Hz, 2H, Ar-H), 7.40 (d, J = 9.1 Hz, 2H, Ar-H), 7.80-8.20 (m, 8H, Ar-H); MS: m/z 576 (M^+). Anal. calcd for $C_{31}H_{20}N_4O_8$: C, 64.58; H, 3.50; N, 9.72. Found: C, 64.11; H, 3.37; N, 9.70.

2-[3-(2-Chlorophenyl)-5-isoxazolyl]-4-{3-[3-(2-chlorophenyl)-5-isoxazolyl]-4-hydroxybenzyl}phen- ol (4g). Yield 83%; mp 118-120 °C; IR (KBr): 3340, 3037, 1594, 1475, 1030, 676 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.98 (s, 2H, CH_2), 4.54 (s, 2H, OH), 6.19 (s, 2H, Ar-H), 6.72 (s, 2H, Ar-H), 7.06 (d, J = 9.1 Hz, 2H, Ar-H), 7.30-7.70 (m, 10H, Ar-H); MS: m/z 555 (M^+). Anal. calcd for $C_{31}H_{20}Cl_2N_2O_4$: C, 67.04; H, 3.63; N, 5.04. Found: C, 66.83; H, 3.71; N, 5.01.

4-{4-Hydroxy-3-[3-(2-hydroxyphenyl)-5-isoxazolyl]benzyl}-2-[3-(2-hydroxyphenyl)-5-isoxazolyl]phe- nol (4h). Yield 85%; mp 172-174 °C; IR (KBr): 3410, 3065, 1595, 1470, 1030 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.98 (s, 2H, CH_2), 6.19 (s, 2H, Ar-H), 6.72 (s, 2H, Ar-H), 7.10 (d, J = 9.1 Hz, 2H, Ar-H), 6.80-7.80 (m, 14H,

Ar-H + OH); MS: m/z 518 (M^+). Anal. calcd for $C_{31}H_{22}N_2O_6$: C, 71.81; H, 4.28; N, 5.40. Found: C, 72.00; H, 4.16; N, 5.36.

2-[3-(2-Bromophenyl)-5-isoxazolyl]-4-{3-[3-(2-bromophenyl)-5-isoxazolyl]-4-hydroxybenzyl}phen- ol (4i). Yield 82%; mp 201-203 °C; IR (KBr): 3064, 1597, 1472, 1035, 580 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.99 (s, 2H, CH_2), 4.53 (s, 2H, OH), 6.19 (s, 2H, Ar-H), 7.10 (d, J = 9.1 Hz, 2H, Ar-H), 7.20-7.80 (m, 12H, Ar-H) ; MS: m/z 644 (M^+). Anal. calcd for $C_{31}H_{20}Br_2N_2O_4$: C, 57.79; H, 3.13; N, 4.35. Found: C, 56.72; H, 3.18; N, 4.25.

2-[3-(2-Furyl)-5-isoxazolyl]-4-{3-[3-(2-furyl)-5-isoxazolyl]-4-hydroxybenzyl}phenol (4j). Yield 83%; mp 196-198 °C; IR (KBr): 3354, 3067, 1595, 1474, 1035 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.99 (s, 2H, CH_2), 4.53 (s, 2H, OH), 6.19 (s, 2H, Ar-H), 6.32 (m, 2H, Ar-H), 6.90-7.10 (m, 8H, Ar-H), 7.40 (d, J = 9.1 Hz, 2H, Ar-H); MS: m/z 466 (M^+). Anal. calcd for $C_{27}H_{18}N_2O_6$: C, 69.53; H, 3.89; N, 6.01. Found: C, 69.33; H, 3.91; N, 5.98.

Acknowledgements

The authors are grateful to the Director, Indian Institute of Chemical Technology, Hyderabad, India for spectral analysis.

References

1 J. U. Yuhong, R. S. Varma, J. Org. Chem. 71, 135 (2006).

2 (a) M. Kawamoto, T. Ikeda, A. Mori, A. Sekiguchi, K. Masui, T. Shimoda, M. Horie, M. Osakada, J. Am. Chem. Soc., 125, 1700 (2003). (b) K. Masui, H. Ikegami, A. Mori, J. Am. Chem. Soc., 126, 5074 (2004). (c) K. Masui, A. Mori, K. Okamo, K. Takamula, M. Kinoshita, T. Ikeda, Org. Lett. 6, 2001 (2004). (d) J. S. Yadav, B. V. S. Reddy, G. Sathis, P. Nagalaxmi, S. Kiran, A. C. Kunwar, Tetrahedron Lett. 45, 8587 (2004). (e) D. A. Maurizio, R. Rocco, Lett. Org. Chem. 1, 12 (2004). (f) A. Mori, K. Kobayashi, M. Ahmed, S. F. Mohamed, Org. Lett. 7, 4487 (2005). (g) G. Jagath Reddy, D. Manjula, K. Srinivas Rao, Indian J. Chem. 44B, 2412 (2005).

3 (a) C. A. Zificsak, D. J. Hlasta, Tetrahedron, 60, 8991 (2004). (b) T. Haino, M. Tanaka, K. Ikeda, K. Kubo, A. Mori, Y. Fukazawa, Tetrahedron Lett. 45, 2277 (2004).

4 K. Tomita, H. Oko, J. Murakami, H. Tsuji, K. Matsumoto, Sankyo Kenkyusho Nempo, 30, 193 (1978).

5 (a) Gaziz M. H. Hosseir, N. L. Doss, A. S. Rock, Indian J. Chem. 14B, 286 (1976). (b) A. Witezak, M. Krolikowaska, Pol. J. Chem. 55, 89 (1981). (c) S. B. Lohiya, B. J. Ghiya, Indian J. Chem. 25B, 279 (1986).

6 (a) V. Padmavathi, R. P. Sumathi, N. Chandra Sekhar Babu, D. Bhaskar Reddy, J. Chem. Res (s). 610 (1999). (b) D. Bhaskar Reddy, A. Somasekhar Reddy, A. Padmaja, Synth. Commun. 29, 4433 (1999). (c) V. Padmavathi, R. P. Sumathi, K. Venugopal Reddy, A. Somasekhar Reddy, D. Bhaskar Reddy, Synth. Commun. 30, 4007 (2000).

7 (a) Claisen, Ber. 36, 3664 (1903). (b) Claisen, Ber. 42, 59 (1909).

8 V. Padmavathi, B. Jagan Mohan Reddy, A. Balaiah, K. Venugopal Reddy, D. Bhaskar Reddy, Molecules, 5, 1281 (2000).

9 (a) Ch. Sanjeeva Reddy, G. Smita, Synth. Comun. 21, 3997 (2004). (b) Ch. Sanjeeva Reddy, G. Smitha, Synth. Commun. 36, 1795 (2006). (c) Ch. Sanjeeva Reddy, A. Nagaraj, Heterocycl. Commun. 13, 67 (2007). (d) Ch. Sanjeeva Reddy, A. Nagaraj, Chin. Chem. Lett. 18, 1431 (2007). (e) Ch. Sanjeeva Reddy, A. Nagaraj, P. Jalapathi, Chin. Chem. Lett. 18, 1213 (2007). (f) Ch. Sanjeeva Reddy, A. Nagaraj, J. Heterocyclic Chem. 44, 1357 (2007). (g) Ch. Sanjeeva Reddy, A. Nagaraj, J. Heterocyclic Chem. 44, 1181 (2007). h) Ch. Sanjeeva Reddy, A. Nagaraj, Indian J. Chem. 47B, 319 (2008). (i) Ch. Sanjeeva Reddy, A. Nagaraj, J. Iran. Chem. Soc. 5, 262 (2008). (j) Ch. Sanjeeva Reddy, A. Srinivas, A. Nagaraj, Indian J. Chem. 47B, 787 (2008). (k) Ch. Sanjeeva Reddy, A. Srinivas, A. Nagaraj, J. Heterocyclic Chem. 45 (2008, Jul-Aug). (l) Ch. Sanjeeva Reddy, A. Srinivas, A. Nagaraj, J. Heterocyclic Chem. 45 (2008, Jul-Aug). (m) Ch. Sanjeeva Reddy, M. Raghu, A. Nagaraj, J. Heterocyclic Chem. 45 (2008, Jul-Aug). (n) Ch. Sanjeeva Reddy, A. Nagaraj, Indian J. Chem. 47B (2008, Jul).

10 C. S. Marvel, N. Tarkoy, J. Am. Chem. Soc., 79, 6001 (1957).

11 J. G. Horsfall, Bot. Rev., 11, 357 (1945).

Received on April 17,2008