Synthesis and *In Vitro* Study of a New Class of Methylene-bis-4,6-diarylbenzo[*d*]isoxazoles as Potential Antifungal Agents

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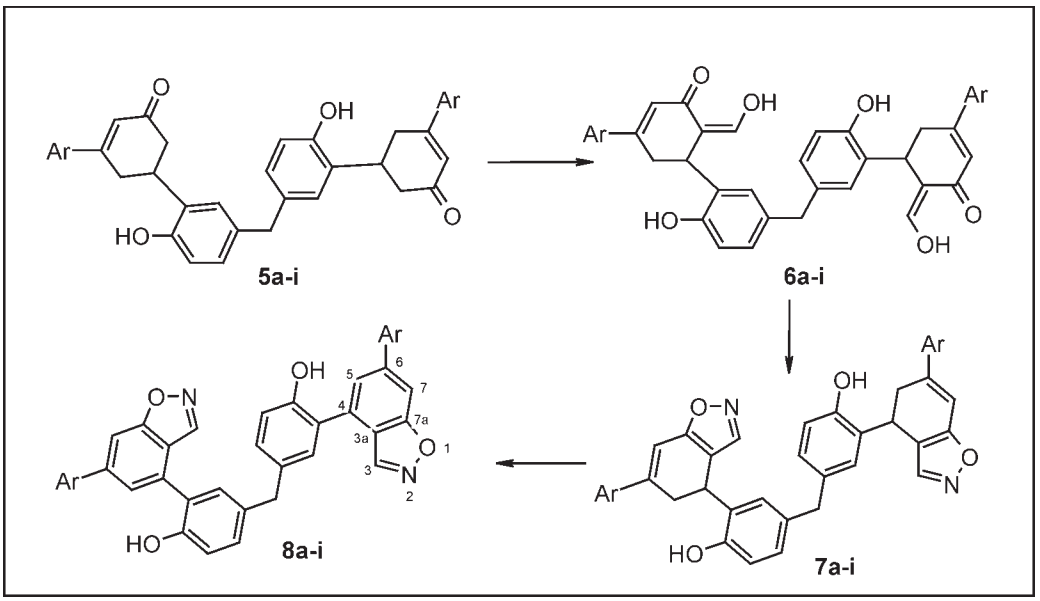

A new class of methylene-bis-4,6-diarylbenzo[*d*]isoxazoles **8a–i** was synthesized by the reaction of methylene-bis-aryl-6-hydroxymethylene-2-cyclohexenone **6** with hydroxylamine hydrochloride, followed by aromatization with DDQ. Chemical structures of the newly synthesized compounds were elucidated by their IR, ^{1}H NMR, ^{13}C NMR, MS, and elemental analyses. Furthermore, all the compounds were screened for their antifungal activity against various fungi and compared with their monomeric compounds. Among the synthesized compounds, **8b**, **8g**, and **8i** were found to be the most active against *Candida albicans* (ATCC 10231), *Aspergillus fumigatus* (HIC 6094), *Trichophyton rubrum* (IFO 9185), and *Trichophyton mentagrophytes* (IFO 40996). It is interesting to note that the compounds **8a**, **8g**, and **8i** showed fungicidal activity toward *C. albicans* at the concentration of 3.12 μg/mL, which is less than the concentration of standard Amphotericin B.

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INTRODUCTION

The treatment of infectious diseases still remains an important and challenging problem because of a combination of factors including emerging infectious diseases and the increasing number of multidrug resistant microbial pathogens. In spite of a large number of antibiotics and chemotherapeutics available for medical use, the emergence of old and new antibiotic resistance created in the last decades revealed a substantial medical need for new classes of antimicrobial agents. There is a real perceived need for the discovery of new compounds endowed with antimicrobial activity, which are distinct from those of well known classes of antibacterial agents to which many clinically relevant pathogens are now resistant. Similarly in recent decades, an increased incidence of fungal infections has been observed as a consequence of the growing number of immunocompromised patients and the frequent use of antibacterial and cytotoxic drugs. For many fungal infections, polyenes, such as Amphotericin B, represent the standard therapy. Polyenes bind to membrane sterols, leading to membrane permeability, leakage, and cell death. However, the clinical use of Amphotericin B is limited by a high frequency of renal toxicity and several adverse effects [1]. Although the various molecules designed and synthesized for the above aim and to reduce the adverse effects, it was demonstrated that isoxazole derivatives could be considered as possible antifungal agents [2]. The other activities include, inhibition of Aβ precursor protein (APP) [3], inhibition of protein tyrosine phosphatase 1B [4], antiviral [5], anthelmintics [6], anti-

inflammatory [7], anticonvulsant [8], insecticidal [9], antitubercular [10], immunomodulatory [11], and hypolipemics [12]. Valdecoxib, an isoxazole derivative, is now widely used in the market as an anti-inflammatory drug [13]. The most general and widely employed synthetic route to isoxazoles involves reaction of chalcones with hydroxylamine hydrochloride [14] or 1,3-dipolar cycloaddition of nitrile oxides to alkynes [15] or condensation of open chain α-hydroxymethylene ketones with hydroxylamine [16] or from 3,5-diarylcyclohexenone and hydroxylamine [17].

Following the successful introduction of antimicrobial agents, inspired by the biological profile of isoxazoles and their increasing importance in pharmaceutical and biological fields, and in continuation of our research on biologically active heterocycles [18], and to enhance the biological activity of isoxazole derivatives, it was considered worth while to synthesize certain new chemical entities incorporating two active pharmacophores in a single molecular framework. In this article, we wish to report the synthesis of a new class of methylene-bis-4,6-diarylbenzo[*d*]isoxazoles **8** in good yields from methylene-bis-aryl-6-hydroxy-methylene-2-cyclohexenone **6** (Scheme 1) and *in vitro* antifungal activity.

RESULTS AND DISCUSSION

The key intermediate, **6** required for the synthesis of the title compounds was prepared according to the procedure outlined in the Scheme 1. Condensation of the salicylaldehyde **1** and trioxane in the presence of a mixture of conc. sulfuric acid and acetic acid gave methylene-bis-salicylaldehyde **2** in good yield [19]. Compound **2** on reaction with the aromatic/heteroaromatic methyl ketones in the presence of alc. KOH at room temperature gave methylene-bis-chalcones **3** (yield over 90%) [18(g)], the reaction time as well as the product yield varies depending on the corresponding reagents. The crude product, contaminated by some starting materials, was purified by extracting with ether. Knoevenagel condensation of compound **3** with ethyl acetoacetate gave methylene-bis-aryl-6-carbethoxycyclohexenones **4** (yield over 80%). Decarboxylation of compound **4**, in the presence of HCl/AcOH at reflux temperature, resulted in methylene-bis-arylcyclohexenone **5** (yield over 80%), which on Claisen-like condensation with ethylformate in the presence of sodium methoxide at room temperature afforded methylene-bis-aryl-6-hydroxymethylene-2-cyclo-hexenone **6** in good yields. Compound **6** on

Scheme 1. Reagents and conditions: (i) trioxane, H_2SO_4/AcOH, reflux, 81%; (ii) $ArCOCH_3$, KOH/EtOH, rt, 82–95%; (iii) EAA, NaOEt/EtOH, reflux, 78–86%; (iv) HCl/AcOH, reflux, 74–82%; (v) HCOOEt, NaOMe/C_6H_6, rt, 79–88%; (vi) $H_2NOH{\cdot}HCl$, AcOH, reflux, 77–86%; and (vii) DDQ/dry C_6H_6, N_2–atm, 76–83%.

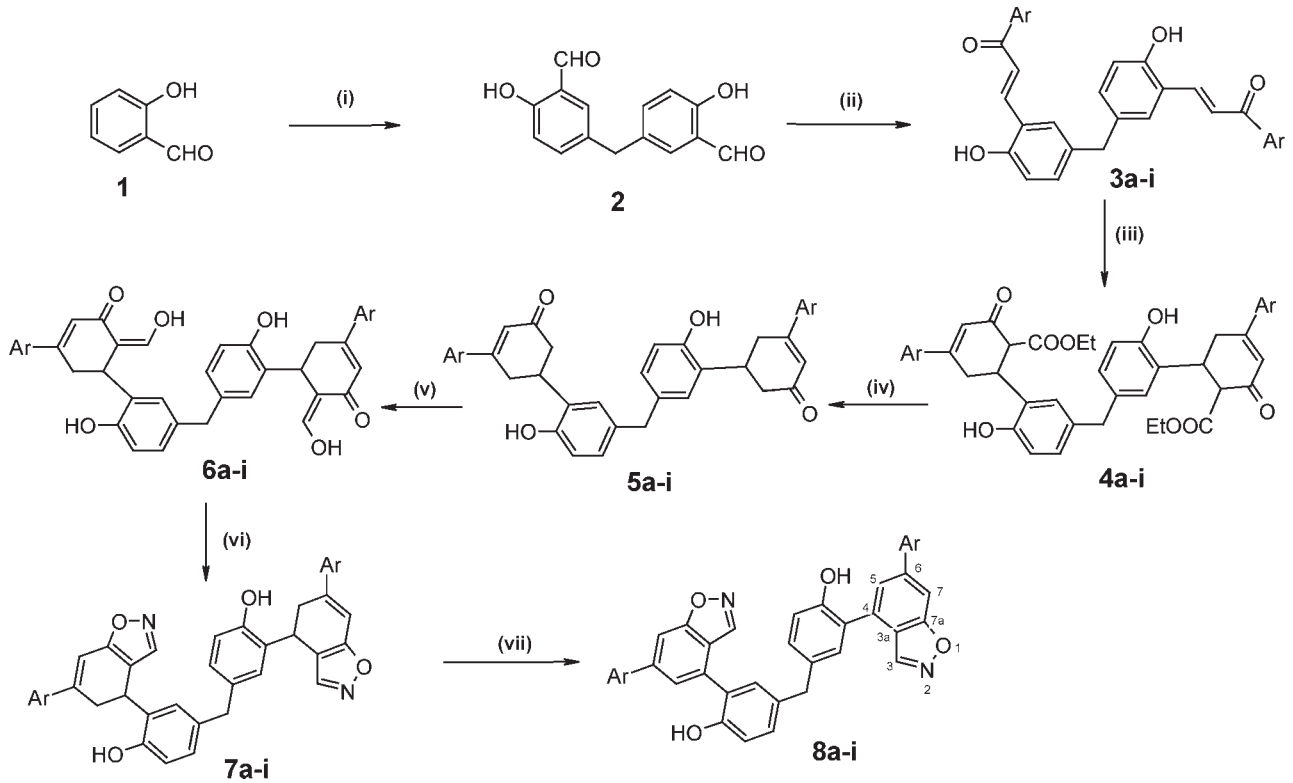
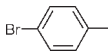
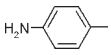
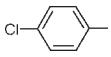
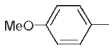


Table 1

Synthesis of compounds **8a–i**.

Product[a]	Ar	Mol. formula	Yield (%)[b]	M.p.°C
8a		$C_{39}H_{26}N_2O_4$	78	158–60
8b		$C_{39}H_{24}Br_2N_2O_4$	84	171–72
8c		$C_{39}H_{28}N_4O_4$	79	158–60
8d		$C_{39}H_{24}Cl_2N_2O_4$	86	144–46
8e		$C_{41}H_{30}N_2O_6$	77	135–37
8f		$C_{35}H_{22}N_2O_6$	80	162–63
8g		$C_{33}H_{20}N_4O_4S_2$	82	149–50
8h		$C_{37}H_{24}N_4O_4$	77	151–53
8i		$C_{35}H_{22}N_6O_4$	81	162–64

[a] All the products were characterized by IR, NMR, MS, and elemental analyses.
[b] Isolated yields after purification.

cyclocondensation with hydroxylamine hydrochloride in refluxing acetic acid gave dihydroisoxazole derivative **7**, in good to excellent yields (yields over 80%). Subsequent aromatization of **7** with dichlorodicyanoparabenzoquinone (DDQ), under N_2-atomsphere at reflux temperature, gave methylene-bis-4,6-diarylbenzo[*d*]isoxazoles **8** in good yields (Table 1). In the ^{1}H NMR spectra of **8**, the absence of signals corresponding to methine and methylene protons of cyclohexadiene ring indicates that aromatization has indeed taken place. The structure of all the synthesized compounds was confirmed by their IR, ^{1}H NMR, ^{13}C NMR, MS, and elemental analyses. Furthermore, the compounds was subjected to antifungal testing and compared with their monomeric compounds prepared from the literature method [17].

In this work, a series of nine new benzo-[*d*]isoxazole derivatives were synthesized and the Scheme 1 illustrates the strategy used for the synthesis of target compounds. Salicylaldehyde and trioxane was used as starting materials to prepare isoxazole derivatives. In the IR spectra of compounds **8a–i**, the C═N and N─O bands of the isoxazole moiety was observed at about 1600 and 1470 cm^{-1}, respectively. In the ^{1}H NMR spectra of compounds **8a–i** recorded in DMSO-d_6, the signal due to methylene bridge protons appeared at 3.98–4.06 ppm as a singlet and the N═CH proton appeared at 8.20–8.28 ppm as a singlet, proved that these compounds have isoxazole nucleus. All the other aromatic and aliphatic protons of **8a–i** were observed at the expected regions. In the ^{13}C NMR spectra of compounds **8a–i** that are taken in DMSO-d_6, the prominent signal corresponding to C-3, C-3a, and C-7a observed at 150.3, 128.0–120.5, 173.0–170.0 ppm, respectively, have proved for further evidence for their structures. Mass spectra of all the synthesized compounds showed M^+/M^++1 peaks are in agreement with their molecular formulae.

Antifungal activity. The newly prepared compounds were screened for their antifungal activity against four fungal organisms viz. *Candida albicans* (ATCC 10231), *Aspergillus fumigatus* (HIC 6094), *Trichophyton rubrum* (IFO 9185), and *Trichophyton mentagrophytes* (IFO 40996) by the broth dilution method, recommended by National Committee for Clinical Laboratory Standards (NCCLs) [20]. The *C. albicans* was grown for 48 h at 28°C in YPD broth (1% yeast extract, 2% peptone, and 2% dextrose), harvested by centrifugation, and then washed twice with sterile distilled water. *A. fumigatus*, *T. rubrum*, and *T. mentagrophytes* were plated in potato dextrose agar (PDA) (Difco) and incubated at 28°C for 2 weeks. Spores were washed three times with sterile distilled water and resuspended in distilled water to obtain an initial inoculum size of 10^5 spores/mL. Each test compound was dissolved in DMSO and diluted with potato dextrose broth (Difco) to prepare serial twofold dilutions in the range 100 to 0.8 μg/mL. Ten microliters of the broth containing about 10^3 (for yeast) and 10^4 (for filamentous fungi) cells/mL of test fungi was added to each well of a 96-well microtiter plate. Culture plates were incubated for ~48–72 h at 28°C. The antifungal activity of each compound was compared with the standard drug Amphotericin B. Minimum inhibitory concentration (MIC, μg/mL) was measured and compared with controls; the MIC values of the compounds screened are presented in Table 2.

The antifungal screening data showed only moderate activity of the tested compounds. Among the screened compounds, **8b**, **8g**, and **8i** in which isoxazole moiety bearing 4-bromophenyl, 5-thiazole, and 2-pyrazine nucleus, respectively, were showed high activity against all the microorganisms employed. The activities of these three compounds are almost equal to the standard. It is

Table 2

Antifungal activity of compounds **8a–i**.

Compd.	Minimum inhibitory concentration (MIC, μg/mL)			
	Candida albicans	*Aspergillus fumigatus*	*Trichophton rubrum*	*Trichophyton mentagrophytes*
8a	25.0[a] (50.0)[b]	50.0[a] (—)[b]	25.0[a] (—)[b]	50.0[a] (—)[b]
8b	3.12 (12.5)	3.12 (12.5)	3.12 (12.5)	3.12 (12.5)
8c	12.5 (12.5)	25.0 (25.0)	25.0 (50.0)	6.25 (12.5)
8d	6.25 (25.0)	25.0 (50.0)	— (—)	12.5 (50.0)
8e	12.5 (25.0)	12.5 (25.0)	6.25 (—)	6.25 (25.0)
8f	25.0 (50.0)	12.5 (25.0)	6.25 (25.0)	6.25 (25.0)
8g	3.12 (25.0)	3.12 (12.5)	3.12 (6.25)	3.12 (12.5)
8h	12.5 (50.0)	3.12 (6.25)	3.12 (25.0)	3.12 (6.25)
8i	3.12 (25.0)	3.12 (12.5)	3.12 (12.5)	3.12 (12.5)
Amphotericin B	6.25	3.12	3.12	3.12

— Indicates fungi are resistant to the compound >50 μg/mL conc.
[a] Activity of dimeric compounds.
[b] Activity of their monomeric compounds.

also interesting to note that the compounds **8b**, **8g**, and **8i** showed activity toward *C. albicans* at the concentration of 3.12 μg/mL, which is less than the concentration of the standard Amphotericin B. Similarly, compound **8h** in which isoxazole ring bearing 2-pyridine nucleus, also showed good antifungal activity equal to the activity of the standard but only toward *A. fumigatus* and *T. rubrum*. The remaining compounds showed moderate to good antifungal activity. Furthermore, the activity of dimeric compounds was compared with that of their monomeric compounds prepared from the reported method [17]. The results reveal that almost all the dimeric compounds showed enhanced activity than their monomeric compounds (Table 2).

In conclusion, a new class of methylene-bis-4,6-diarylbenzo[*d*]isoxazoles **8a–i** has been designed and synthesized. The antifungal activity of these compounds was evaluated against various fungi. Among the synthesized compounds, **8b**, **8g**, and **8i** showed good activity against test fungi and emerged as potential molecules for further development. With this set of analogues, we are now in a position to investigate the multiple biological activities for these compounds.

EXPERIMENTAL

All the chemicals and solvents were of analytical grade and used as purchased. A proper safety measure was taken while carring out the reactions. Evaporations were performed at reduced pressure below 40°C. The reactions and purifications were monitored by TLC on aluminium sheets coated with silica gel 60 F_{254} (Merck), column chromatography on silica gel 60 (Merck). Melting points were taken using a Fisher-Johns melting point instrument and are uncorrected. IR spectra were obtained on a Perkin-Elmer FTIR 5000 spectrometer, using KBr pellets. ^{1}H NMR and ^{13}C NMR spectra were obtained with Varian Gemini (^{1}H: 300 MHz, ^{13}C: 75 MHz) spectrometer, and the chemical shifts were reported as parts per million (δ ppm) down field from internal tetramethylsilane and coupling constants (*J*) in Hz. Mass spectra were obtained on a VG Micromass 7070H spectrometer. Elemental analyses were performed on a Perkin-Elmer 240 CHN elemental analyzer.

Ethyl-6-(5-{3-[6-(ethoxycarbonyl)-5-oxo-3-phenyl-3-cyclohexenyl]-4-hydroxybenzyl}-2-hydroxyphenyl)-2-oxo-4-phenyl-3-cyclohexene-1-carboxylate (4a). To a solution of sodium metal (2 g) in ethanol (30 mL), a mixture of freshly distilled ethylacetoacetate (3.9 mL, 0.03 mol) and compound **3a** (4.6 g, 0.01 mol) dissolved in ethanol (20 mL) was added. The resulting solution was refluxed on a water bath for 4 h. Allowing the reaction mixture to cool and crystallization of the formed precipitate from ethanol gave **4a** (82% yield) as brown solid; mp 150–152°C; ^{1}H NMR (DMSO-d_6): δ 1.10 (6H, t, CH_3), 2.87 (4H, d, CH_2), 3.72 (2H, s, CH_2), 3.81 (2H, d, CH), 3.87 (2H, q, CH), 4.06 (4H, q, CH_2), 5.20 (2H, s, OH), 6.10 (2H, s, CH), 6.62 (2H, d, J = 9.2 Hz, ArH), 6.79 (2H, d, J = 9.2 Hz, ArH), 6.80 (2H, s, ArH), 7.10–7.14 (10H, m, ArH); ^{13}C NMR (DMSO-d_6): δ 17.0, 30.7, 37.0, 42.1, 60.6, 61.2, 117.4, 121.9, 123.8, 125.5, 127.9, 128.2, 128.7, 130.0, 133.4, 142.3, 149.5, 154.6, 176.7, 190.1; IR (KBr): υ 3452, 3065, 1702, 1695, 1597, 1245 cm^{-1}; MS: *m*/*z* 685 (M^+ +1). The other compounds **4b–i** were also prepared by the similar procedure.

5-{2-Hydroxy-5-[4-hydroxy-3-(5-oxo-3-phenyl-3-cyclohexenyl)benzyl]phenyl-3-phenyl}-2-cyclohexen-1-one (5a). To a mixture of glacial AcOH (100 mL) and conc. HCl (50 mL) was added compound **4a** (6.5 g, 0.01 mol) in portions. The mixture was heated to reflux for 10 h. After cooling to room temperature, the reaction mixture was concentrated *in vacuo*. The residue was taken up with ethyl acetate and washed with water and brine, dried over $MgSO_4$, filtered, and evaporated *in vacuo* to give oil, which soon solidified. It was purified by recrystallization from ethanol to give compound **5a** (79% yield) as brown solid; mp 132–134°C; ^{1}H NMR (DMSO-d_6): δ 2.67 (4H, d, CH_2), 2.70 (4H, d, CH_2), 3.72 (2H, s, CH_2), 3.83 (2H, m, CH), 5.20 (2H, s, OH), 6.10 (2H, s, CH), 6.62 (2H, d, J = 9.2 Hz,

ArH), 6.82 (2H, s, ArH), 7.00 (2H, d, J = 9.2 Hz, ArH), 7.10–7.14 (10H, m, ArH); ^{13}C NMR (DMSO-d_6): δ 31.0, 38.9, 42.0, 47.5, 116.1, 122.6, 124.5, 126.2, 127.4, 127.8, 128.5, 131.2, 132.3, 140.5, 150.1, 155.2, 198.0; IR (KBr): υ 3357, 3025, 2932, 1687, 1596 cm^{-1}; MS: m/z 541 (M^+ +1). The other compounds **5b–i** were also prepared by the similar procedure.

5-[2-Hydroxy-5-(4-hydroxy-3-{6-[(*Z*)-1-hydroxymethylidene]-5-oxo-3-phenyl-3-cyclohexenyl}benzyl)phenyl]-6-[(*Z*)-1-hydroxymethylidene]-3-phenyl-2-cyclohexen-1-one (6a). In a solution of 10% sodium methoxide (10 mL) in benzene (25 mL), ethylformate (2.24 mL, 0.03 mol) was added and then compound **5a** (5.4 g, 0.01 mol), dissolved in benzene (10 mL), was added over 30 min. The resulting solution was stirred for 10 h at room temperature and allowed to stand overnight, then evaporated to dryness. The suspension obtained was mixed with cold water, acidified with dil HCl (20 mL), and extracted three times with ether (40 mL). The organic layer was dried over $MgSO_4$, evaporated *in vacuo* to give solid, and purified by crystallization in ethanol to afford pure 6a (81% yield) as yellow solid; mp 143–145°C; ^{1}H NMR (DMSO-d_6): δ 3.22 (4H, d, CH_2), 3.72 (2H, s, CH_2), 4.12 (2H, t, CH), 5.67 (2H, s, CH), 6.40–6.49 (4H, m, ArH), 6.80 (2H, s, ArH), 7.10–7.14 (10H, m, ArH), 7.92 (2H, s, CH), 8.97 (2H, s, OH); ^{13}C NMR (DMSO-d_6):δ 37.8, 42.0, 44.1, 115.5, 116.7, 126.2, 127.6, 127.9, 128.0, 128.9, 130.2, 130.6, 131.1, 142.0, 149.7, 156.1, 167.6, 191.3; IR (KBr): υ 3320, 3028, 2952, 1662, 1620, 1597 cm^{-1}; MS: m/z 596 (M^+). The other compounds **6b–i** were also prepared by the similar procedure.

4-[4-Hydroxy-3-(6-phenyl-4,5-dihydrobenzo[*d*]isoxazol-4-yl)benzyl]-2-(6-phenyl-4,5-dihydrobenzo[*d*]isoxazol-4-yl)phenol (7a). To a solution of 6a (5.9 g, 0.01 mol) in glacial acetic acid (50 mL), hydroxylamine hydrochloride (2.0 g, 0.03 mol) was added. After stirring at 80°C for 10 h, the mixture was concentrated *in vacuo*. To the residue was added water and twice extracted with ether. The organic layer was washed with saturated $NaHCO_3$ solution, subsequently with water and birne, dried over $MgSO_4$ and evaporated to dryness. The residue was recrystallized from ethanol to afford 7a (79% yield) as brown solid; mp 123–125°C; ^{1}H NMR (DMSO-d_6): δ 2.82 (4H, d, CH_2), 3.72 (2H, s, CH_2), 4.22 (2H, t, CH), 4.62 (2H, s, OH), 6.70 (2H, s, ArH), 6.73 (2H, d, J = 9.0 Hz, ArH), 6.84 (2H, d, J = 9.1 Hz, ArH), 6.99 (4H, m, ArH), 7.00 (2H, s, ArH), 7.21 (2H, s, ArH), 7.32 (4H, d, J = 9.2 Hz, ArH); ^{13}C NMR (DMSO-d_6): δ 38.1, 39.3, 42.1, 110.5, 117.2, 118.7, 125.4, 126.5, 127.8, 128.0, 128.9, 130.0, 132.0, 132.8, 140.3, 143.4, 156.2, 161.7; IR (KBr): 3390, 3037, 2972, 1609, 1470, 1030 cm^{-1}; MS: m/z 590 (M^+). The other compounds **7b–i** were also prepared by the similar procedure.

4-[4-Hydroxy-3-(6-phenylbenzo[*d*]isoxazol-4-yl)benzyl]-2-(6-phenylbenzo[*d*]isoxazol-4-yl)phenol (8a). To a solution of **7a** (5.9 g, 0.01 mol) in dry benzene (20 mL), DDQ (6.81 g, 0.03 mol), dissolved in dry benzene (20 mL), was added in portions. The mixture was heated to reflux and stirred for 5 h under nitrogen atmosphere. The precipitated DDQ-H_2 was filtered off and the filtrate was subjected to column chromatography on silica gel (60–120 mesh) to afford pure **8a** (78% yield) as orange solid; mp 158–160°C; ^{1}H NMR (DMSO-d_6): δ 3.99 (2H, s, CH_2), 4.65 (2H, s, OH), 6.90 (2H, s, ArH), 6.99 (2H, d, J = 8.9 Hz, ArH), 7.29 (2H, d, J = 8.9 Hz, ArH), 7.44–7.50 (10H, m, ArH), 7.90 (2H, s, ArH), 8.20 (2H, s, ArH), 8.60 (2H, s, ArH); ^{13}C NMR (DMSO-d_6): δ 40.7, 115.1, 117.0, 125.4, 127.0, 127.9, 128.3, 130.0, 133.2, 134.7, 137.4, 144.9, 145.2, 150.4, 151.2, 153.6, 159.3, 171.4; IR (KBr): υ 3344, 3062, 2972, 1609, 1470, 1030 cm^{-1}; MS: m/z 586 (M^+). Anal. calcd. for $C_{39}H_{26}N_2O_4$: C, 79.85; H, 4.47; N, 4.78. Found: C, 79.90; H, 4.39; N, 4.71. The other compounds **8b–i** were also prepared by the similar procedure.

2-[6-(4-Bromophenyl)benzo[*d*]isoxazol-4-yl]-4-3-[6-(4-bromophenyl)benzo[*d*]isoxazol-4-yl]-4-hydroxybenzylphenol (8b). This compound was obtained as brown solid; yield 84%; mp 171–172°C; ^{1}H NMR (DMSO-d_6): δ 4.00 (2H, s, CH_2), 4.65 (2H, s, OH), 6.90 (2H, s, ArH), 7.00 (2H, d, J = 8.9 Hz, ArH), 7.29 (2H, d, J = 8.9 Hz, ArH), 7.40–7.45 (8H, m, ArH), 7.90 (2H, s, ArH), 8.20 (2H, s, ArH), 8.60 (2H, s, ArH); ^{13}C NMR (DMSO-d_6): δ 40.7, 115.0, 117.1, 122.1, 125.4, 130.0, 130.6, 132.3, 133.1, 134.6, 137.3, 141.3, 144.9, 147.1, 151.3, 153.6, 159.4, 171.4; IR (KBr): υ 3390, 3065, 2995, 1609, 1470, 1030, 586 cm^{-1}; MS: m/z 742/744/746 (M^+). Anal. calcd. for $C_{39}H_{24}Br_2N_2O_4$: C, 62.92; H, 3.25; N, 3.76. Found: C, 62.85; H, 3.30; N, 3.70.

2-[6-(4-Aminophenyl)benzo[*d*]isoxazol-4-yl]-4-3-[6-(4-aminophenyl)benzo[*d*]isoxazol-4-yl]-4-hydroxybenzylphenol (8c). This compound was obtained as brown solid; yield 79%; mp 158–160°C; ^{1}H NMR (DMSO-d_6): δ 4.06 (2H, s, CH_2), 4.65 (2H, s, OH), 6.68 (4H, d, J = 8.5 Hz, ArH), 6.90 (2H, s, ArH), 7.00 (2H, d, J = 8.9 Hz, ArH), 7.31 (2H, d, J = 8.9 Hz, ArH), 7.52 (4H, d, J = 8.5 Hz, ArH), 7.90 (2H, s, ArH), 8.20 (2H, s, ArH), 8.60 (2H, s, ArH); ^{13}C NMR (DMSO-d_6): δ 40.7, 115.9, 116.7, 125.3, 127.3, 128.5, 130.1, 133.0, 134.1, 134.7, 137.5, 139.6, 144.5, 146.3, 151.2, 153.4, 159.2, 171.3; IR (KBr): υ 3390, 3065, 2972, 1612, 1469, 1028 cm^{-1}; MS: m/z 616 (M^+). Anal. calcd. for $C_{39}H_{28}N_4O_4$: C, 75.96; H, 4.58; N, 9.09. Found: C, 75.85; H, 4.60; N, 9.03.

2-[6-(4-Chlorophenyl)benzo[*d*]isoxazol-4-yl]-4-3-[6-(4-chlorophenyl)benzo[*d*]isoxazol-4-yl]-4-hydroxybenzylphenol (8d). This compound was obtained as yellow solid; yield 86%; mp 144–146°C; ^{1}H NMR (DMSO-d_6): δ 4.02 (2H, s, CH_2), 4.68 (2H, s, OH), 6.90 (2H, s, ArH), 7.00 (2H, d, J = 8.9 Hz, ArH), 7.31 (2H, d, J = 8.9 Hz, ArH), 7.39 (4H, d, J = 8.1 Hz, ArH), 7.83 (4H, d, J = 8.1 Hz, ArH), 7.90 (2H, s, ArH), 8.22 (2H, s, ArH), 8.51 (2H, s, ArH); ^{13}C NMR (DMSO-d_6): δ 40.7, 115.7, 117.2, 125.4, 127.3, 128.5, 130.1, 133.0, 134.1, 134.7, 137.5, 139.6, 144.5, 146.3, 151.2, 153.4, 159.2, 171.3; IR (KBr): υ 3384, 3062, 2968, 1605, 1470, 1028, 782 cm^{-1}; MS: m/z 654/656/658 (M^+). Anal. calcd. for $C_{39}H_{24}Cl_2N_2O_4$: C, 71.46; H, 3.69; N, 4.27. Found: C, 71.42; H, 3.61; N, 4.31.

4-4-Hydroxy-3-[6-(4-methoxyphenyl)benzo[*d*]isoxazol-4-yl]benzyl-2-[6-(4-methoxyphenyl)benzo[*d*]isoxazol-4-yl]phenol (8e). This compound was obtained as yellow solid; yield 77%; mp 135–137°C; ^{1}H NMR (DMSO-d_6): δ 3.81 (6H, s, OMe), 4.02 (2H, s, CH_2), 4.70 (2H, s, OH), 6.90 (2H, s, ArH), 6.96 (4H, d, J = 8.4 Hz, ArH), 7.00 (2H, d, J = 8.9 Hz, ArH), 7.32 (2H, d, J = 8.9 Hz, ArH), 7.34 (4H, d, J = 8.4 Hz, ArH), 7.90 (2H, s, ArH), 8.22 (2H, s, ArH), 8.51 (2H, s, ArH); ^{13}C NMR (DMSO-d_6): δ 40.5, 54.7, 115.0, 112.1, 117.0, 125.0, 129.5, 130.1, 133.1, 134.1, 137.3, 139.5, 144.3, 147.2, 151.1, 153.5, 159.1, 160.7, 172.1; IR (KBr): υ 3384, 3062, 2968, 1605, 1470, 1240 cm^{-1}; MS: m/z 646 (M^+). Anal. calcd. for $C_{41}H_{30}N_2O_6$: C, 76.15; H, 4.68; N, 4.33. Found: C, 76.21; H, 4.61; N, 4.35.

2-[6-(2-Furyl)benzo[*d*]isoxazol-4-yl]-4-3-[6-(2-furyl)benzo-[*d*]isoxazol-4-yl]-4-hydroxybenzylphenol (8f). This compound was obtained as black solid; yield 80%; mp 162–163°C; ^{1}H

NMR (DMSO-d_6): δ 4.02 (2H, s, CH_2), 4.70 (2H, s, OH), 6.34 (2H, m, ArH), 6.82 (2H, s, ArH), 6.70 (2H, m, ArH), 6.96 (2H, d, J = 8.9 Hz, ArH), 7.32 (2H, d, J = 8.9 Hz, ArH), 7.39 (2H, m, ArH), 7.90 (4H, m, ArH), 8.00 (2H, s, ArH), 8.22 (2H, s, ArH); ^{13}C NMR (DMSO-d_6): δ 40.8, 106.2, 106.5, 114.4, 115.1, 121.7, 126.9, 134.1, 138.7, 139.6, 141.9, 143.1, 151.1, 159.1, 159.8, 176.4; IR (KBr): υ 3390, 3071, 2965, 1609, 1470, 1030 cm^{-1}; MS: m/z 566 (M^+). Anal. calcd. for $C_{35}H_{22}N_2O_6$: C, 74.20; H, 3.91; N, 4.94. Found: C, 74.12; H, 3.90; N, 4.83.

4-4-Hydroxy-3-[6-(1,3-thiazol-5-yl)benzo[*d*]isoxazol-4-yl]-benzyl-2-[6-(1,3-thiazol-5-yl)benzo[*d*]isoxazol-4-yl]phenol (8g). This compound was obtained as brown solid; yield 82%; mp 149–150°C; ^{1}H NMR (DMSO-d_6): δ 4.02 (2H, s, CH_2), 4.70 (2H, s, OH), 6.74 (2H, s, ArH), 6.96 (2H, d, J = 8.9 Hz, ArH), 7.32 (2H, d, J = 8.9 Hz, ArH), 7.90–7.95 (4H, m, ArH), 8.10 (2H, s, ArH), 8.22 (2H, s, ArH), 8.62 (2H, s, ArH); ^{13}C NMR (DMSO-d_6): δ 40.6, 105.9, 115.1, 120.1, 120.9, 126.3, 134.0, 137.4, 138.3, 138.9, 140.5, 142.7, 150.9, 151.7, 158.4, 160.3, 173.1; IR (KBr): υ 3390, 3071, 2965, 1609, 1580, 1470, 638 cm^{-1}; MS: m/z 600 (M^+). Anal. calcd. for $C_{33}H_{20}N_4O_4S_2$: C, 65.99; H, 3.36; N, 9.33. Found: C, 65.89; H, 3.40; N, 9.24.

4-4-Hydroxy-3-[6-(2-pyridyl)benzo[*d*]isoxazol-4-yl]benzyl-2-[6-(2-pyridyl)benzo[*d*]isoxazol-4-yl]phenol (8h). This compound was obtained as brown solid; yield 77%; mp 151–153°C; ^{1}H NMR (DMSO-d_6): δ 4.02 (2H, s, CH_2), 4.68 (2H, s, OH), 6.51 (2H, s, ArH), 7.00 (4H, m, ArH), 7.31 (2H, d, J = 8.9 Hz, ArH), 7.59 (2H, m, ArH), 7.72 (2H, m, ArH), 7.92 (2H, s, ArH), 8.22 (2H, s, ArH), 8.62 (2H, d, J = 4.7 Hz, ArH), 8.92 (2H, s, ArH); ^{13}C NMR (DMSO-d_6): δ 40.8, 115.0, 117.0, 121.7, 124.7, 125.3, 130.1, 133.0, 134.0, 135.3, 137.1, 144.1, 144.8, 146.3, 151.1, 153.4, 157.9, 160.3, 169.4; IR (KBr): υ 3390, 3072, 2969, 1605, 1470 cm^{-1}; MS: m/z 588 (M^+). Anal. calcd. for $C_{37}H_{24}N_4O_4$: C, 75.50; H, 4.11; N, 9.52. Found: C, 75.41; H, 4.15; N, 9.47.

4-4-Hydroxy-3-[6-(2-pyrazinyl)benzo[*d*]isoxazol-4-yl]benzyl-2-[6-(2-pyrazinyl)benzo[*d*]isoxazol-4-yl]phenol (8i). This compound was obtained as black solid; yield 81%; mp 162–164°C; ^{1}H NMR (DMSO-d_6): δ 4.02 (2H, s, CH_2), 4.68 (2H, s, OH), 6.72 (2H, s, ArH), 7.00 (2H, d, J = 8.9 Hz, ArH), 7.31 (2H, d, J = 8.9 Hz, ArH), 7.90 (2H, s, ArH), 8.22 (2H, s, ArH), 8.41 (2H, d, J = 2.7 Hz, ArH), 8.64 (2H, d, J = 2.7 Hz, ArH), 8.80 (2H, s, ArH), 8.92 (2H, s, ArH); ^{13}C NMR (DMSO-d_6): δ 40.7, 110.7, 115.1, 127.1, 130.4, 134.0, 137.1, 138.0, 142.1, 143.4, 143.9, 144.1, 144.9, 146.3, 151.2, 156.7, 158.4, 169.4; IR (KBr): υ 3384, 3062, 2968, 1609, 1470, 1028, cm^{-1}; MS: m/z 590 (M^+). Anal. calcd. for $C_{35}H_{22}N_6O_4$: C, 71.18; H, 3.75; N, 14.23. Found: C, 71.11; H, 3.74; N, 14.17.

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