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2-Hydroxyphenacyl azoles and related azolium derivatives as antifungal agents

Saeed Emami,[a,*] Alireza Foroumadi,[b,d] Mehraban Falahati,[c] Ensieh Lotfali,[c] Saeed Rajabalian,[d] Soltan-Ahmed Ebrahimi,[c] Shirin Farahyar[c] and Abbas Shafiee[b]

[a]*Department of Medicinal Chemistry, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari 48175, Iran*
[b]*Department of Medicinal Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran 14174, Iran*
[c]*Department of Parasitology, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran*
[d]*Kerman Neuroscience Research Center, Kerman University of Medical Sciences, Kerman, Iran*

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Abstract—2-Hydroxyphenacyl azole and 2-hydroxyphenacyl azolium compounds have been described as a new class of azole antifungals. Most target compounds showed significant in vitro antifungal activities against tested fungi (*Candida albicans*, *Saccharomyces cerevisiae*, *Aspergillus niger*, and *Microsporum gypseum*) with low MICs values included in the range of 0.25–32 μg/mL comparable to reference drug fluconazole. The most active compounds were also assessed for their cytotoxicity using MTT colorimetric assay on normal mouse fibroblast (NIH/3T3) cells. The results of antifungal activity and toxicity tests indicated that these compounds display antifungal activity at non-cytotoxic concentrations.

The incidence of invasive fungal infections caused by opportunistic pathogens, often characterized by high mortality rates, has been increasing over the past two decades. Patients that become severely immunocompromised because of underlying diseases such as leukemia or, recently, acquired immunodeficiency syndrome or patients who undergo cancer chemotherapy or organ transplantation are particularly susceptible to opportunistic fungal infections.[1,2] A matter of concern in the treatment of fungal infections is the limited number of efficacious antifungal drugs. Many of the currently available drugs are toxic, produce recurrence because they are fungistatic and not fungicides or lead to the development of resistance due in part to the prolonged periods of administration of the available antifungal drugs. There is, therefore, a clear need for the discovery of new structures with antifungal properties, which could lead to the development of new drugs for the management of fungal infections and treatment of systemic mycoses.[3]

Up to only 30 years ago the choice of systemically available antimycotics was between two drugs, amphotericin B and flucytosine, neither of which was satisfactory. Then a series of inhibitors of the biosynthesis of ergosterol, the major sterol of the fungal cell membrane, were found to have excellent antifungal activity, improved safety, and some were also active after oral or parenteral application. Starting with miconazole and ketoconazole, and improving through fluconazole and itraconazole, the imidazole and triazole inhibitors of lanosterol l4α-demethylation have been the most successful. Although the use of a new generation of triazoles, the available polyenes in lipid formulations, the use of echinocandins or the combination therapy have been introduced as alternatives in the last 10 years, the number of available preparations to treat systemic fungal infections is still limited and more alternatives are needed, particularly with improved efficacy against emerging pathogens with limited susceptibility to the available preparations.[4,5]

In order to seek new antifungal agents we synthesized a series of 2-hydroxyphenacyl-azole and 2-hydroxyphenacyl-azolium compounds depicted in Figure 1. We presumed that the azole portion of the molecule is essential to the high antifungal activity. It is a common

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* Corresponding author. E-mail: sd_emami@yahoo.com

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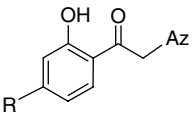


R = H, Cl, OMe
Az = 1*H*-imidazol-1-yl; 1*H*-1,2,4-triazol-1-yl; 4*H*-1,2,4-triazol-4-yl; 1*H*-1,2,4-triazolium; 4-amino-4*H*-1,2,4-triazoliumyl

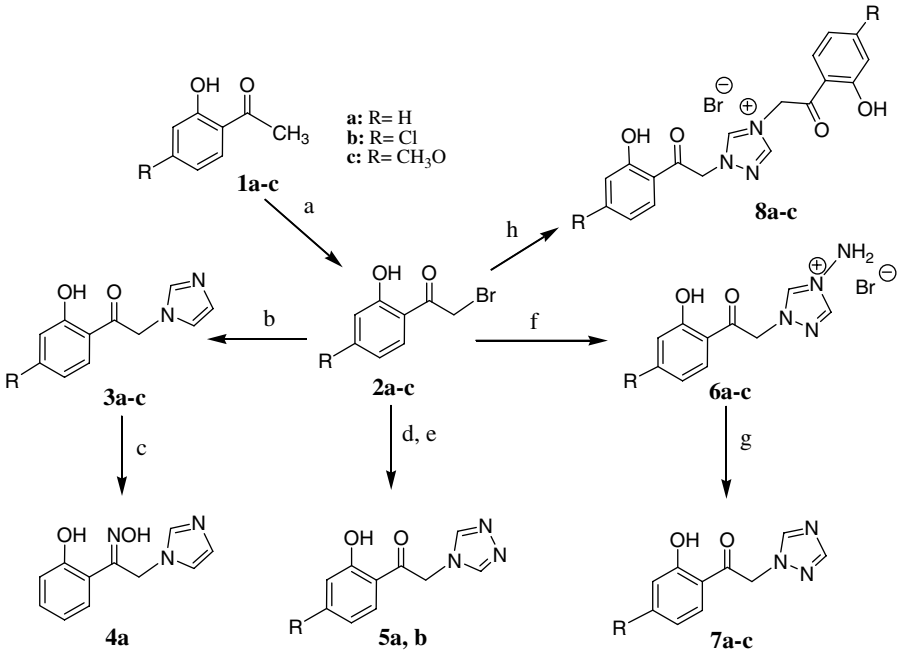
Figure 1. General structure of 2-hydroxyphenacyl azole and 2-hydroxyphenacyl azolium compounds.

substructure seen in many other azole antifungals. The substitution pattern of the azole ring was explored using 1*H*-imidazole, 1,2,4-1*H*-triazole, 1,2,4-4*H*-triazole, 4-aminotriazolium, and 1,4-disubstituted triazolium in the (un)substituted 2-hydroxy acetophenone skeleton (Fig. 1). On the other hand, several studies reveal the importance of phenolic groups for the antifungal activity of acetophenone derivatives such as chalcones against various fungi. In addition, the antifungal activity of these hydroxylated acetophenone derivatives related to the location of the phenolic group in the aryl ring and compounds possessing 2-hydroxyl function showed better activity.[6–9] Accordingly, we wish to report here the synthesis and antifungal activity of 2-hydroxyphenacyl-azole and 2-hydroxyphenacyl-azolium derivatives.

Our synthetic pathway to target compounds **3**–**8** is presented in Scheme 1. The starting 2-hydroxyacetophenones **1** were obtained from the corresponding phenols according to the method reported in the literature.[10] Ketone **1** was brominated with copper (II) bromide in refluxing $CHCl_3$–AcOEt to give corresponding α-bromoketone **2**.[11] Reaction of imidazole with α-bromoketone **2** in DMF, at 40–45 °C, afforded the corresponding imidazole derivatives **3**.[12,13] Compound **3a** was converted to the oxime derivative **4a** by stirring with excess of $HONH_2$·HCl in methanol at room temperature.[14]

Several routes have been developed for the regiospecific synthesis of 1-substituted and 4-substituted 1,2,4-triazoles.[15,16] The thermodynamically less stable regioisomer **5**, can be obtained by blocking the more reactive N-atom of 1,2,4-triazole, followed by quaternization and cleavage of the protecting group.[17] Thus, quaternization of cyanoethyl modified triazole (1*H*-1,2,4-triazole-1-propanenitrile)[17] could be achieved with bromoacetophenones **2** and subsequent treatment with aqueous sodium hydroxide gave the expected product **5**.[18] For preparation of 1-substituted 1,2,4-triazoles, 4-amino-1,2,4-triazole,[15] a protected, directing synthetic equivalent of 1,2,4-triazole can be utilized in cases where regiospecificity under mild nonbasic conditions is required. Thus, the quaternization of 4-amino-1,2,4-triazole with **2** was utilized to form pure triazolium salts **6**,[19] which on subsequent deamination with nitrous acid yielded exclusively the 1-substituted product **7**.[20,21] Compounds **2** were converted to bis(substituted)triazolium bromide derivative **8** by stirring with 0.5 equiv of 1,2,4-triazole in DMF at room temperature.[22]

The in vitro antifungal activities of the synthesized compounds **3**–**8** were investigated against several representative pathogenic fungi as yeast (*Candida albicans* PTCC 5027 and *Saccharomyces cerevisiae* PTCC 5177), dermatophyte (*Microsporum gypseum* PTCC 5070), and mould (*Aspergillus niger* PTCC 5012). The minimum inhibitory concentrations (MICs) were determined by agar dilution method.[23] Sabouraud Dextrose Agar was employed for fungal growth. Stock solutions of tested compounds



Scheme 1. Reagents and conditions: (a) $CuBr_2$, $CHCl_3$–EtOAc, reflux; (b) imidazole, DMF, 40–45 °C; (c) $HONH_2$·HCl, MeOH, rt; (d) 1*H*-1,2,4-triazole-1-propanenitrile, CH_3CN; (e) aq NaOH; (f) 4-amino-1,2,4-triazole, 2-propanol, reflux; (g) $NaNO_2$, HCl; (h) 1*H*-1,2,4-triazole 0.5 equiv, DMF, rt.

were prepared in dimethylsulfoxide (DMSO). Inocula containing approximately 10^6 CFUs/mL of fungi were prepared from broth cultures in log phase growth. Fungal plates were made in triplicate and incubated at 30 °C about 24–48 h for yeast, about 72 h for moulds and about 168 h for dermatophytes. The MIC value was defined as lowest concentration of the antifungal agent at which there was no growth. The MIC values (in μg/mL) for different pathogenic fungi, in comparison with fluconazole and miconazole, are summarized in Table 1.

The results of in vitro antifungal activities showed that the title compounds were active against nearly all fungi tested to some extent. Most derivatives showed significant in vitro antifungal activities against tested fungi with low MIC values included in the range of 0.25–32 μg/mL.

Compound **6c** followed by compound **6b** was the most potent against *C. albicans*, with MIC values of 2 μg/mL and 4 μg/mL, respectively. These compounds possessed superior activity with respect to the reference drug fluconazole (MIC = 8 μg/mL) against this yeast. In addition, methoxy analogs **3c**, **7c**, and **8c** were equivalent in anti-*Candida* activity with MIC values of 8 μg/mL and their activity was found to be comparable to reference drug fluconazole. The MIC values of the test derivatives against *S. cerevisiae* indicate that most compounds possessed a comparable or better activity with respect to fluconazole. In fact, the most active compounds were **3b**, **3c**, and **6b** (MIC = 0.25 μg/mL) being 128-fold more active than fluconazole and four times more active than miconazole against *S. cerevisiae*. Furthermore, compound **8a** with MIC value of 1 μg/mL showed good activity against *S. cerevisiae* comparable to miconazole and 32-fold more potent than fluconazole. Among the compounds tested, compounds **3c**, **5a**, **6c**, **7b**, and **8c** showed highest activity against *A. niger* and their MIC values were determined to be 16 μg/mL, comparable to that of fluconazole. Compounds **3c** and **6c** also inhibited more significantly the growth of *M. gypseum* (MIC = 8 μg/mL) equipotent to miconazole and 4-fold more potent than fluconazole. Moreover, compounds **5a**, **6b**, **7b**, **7c**, **8a** and **8c** showed considerable activity against *M. gypseum* with MIC values of 16 μg/mL.

In general, the results of antifungal evaluation of test compounds in comparison with reference drugs indicated that all methoxy analogs **3c**, **6c**–**8c** along with **6b** and **8a** showed comparable or more potent antifungal activity with respect to the reference drug fluconazole against all tested fungal species.

The compounds **3c**, **6b**, **6c**, **7c**, **8a**, and **8c** that were most active (MIC $\leqslant$ 16 μg/mL) as anti-*Candida* agents were also assessed for their cytotoxicity using MTT colorimetric assay on normal mouse fibroblast (NIH/3T3) cells.[24,25] The IC_{50} values obtained for the six compounds in comparison with reference drug fluconazole are shown in Table 2.

As can be seen from the results, there was no significant cytotoxicity at the concentrations below 47 μg/mL. Among the compounds used, bisphenacyl triazolium derivative **8a** showed greater toxicity with IC_{50} values of 47 ± 5 μg/mL. Also bis(4-methoxyphenacyl)triazolium derivative **8c** showed moderate cytotoxic effect on NIH/3T3 cell line at concentration <100 μg/mL. On the other hand, mono-phenacyl azoles **3c**, **6b**, **6c**, and **7c** showed no significant toxicity at the concentrations used (IC_{50} values = 221–370 μg/mL). The results recorded in Table 2 showed compound **6b** to be significantly less toxic than other compounds and there was no significant deference between the cytotoxicity of compound **6b** and reference drug fluconazole.

In comparison of the results of toxicity and antifungal activity tests, it is seen that these compounds display antifungal activity at non-cytotoxic concentrations thus the antifungal activity of the compounds is not due to their general toxicity effect, however their antifungal activity can be possibly because of their selective antifungal effect.

In terms of structure–activity relationships (SARs), the substitution pattern of azole ring was explored using 1*H*-imidazole, 1,2,4-1*H*-triazole, 1,2,4-4*H*-triazole, 4-aminotriazolium, and 1,4-disubstituted triazolium in (un)substituted 2-hydroxyacetophenone skeleton. Generally, the type of azole ring and chlorine atom linked to the 4-position of the phenacyl moiety seemed to have different influence on the antifungal activity against various fungi strains. Yeasts (*C. albicans* and *S. cerevisiae*), in particular, seemed to be more sensitive toward both changes in the substitution pattern of the azole ring, and phenacyl moiety. In 1*H*-imidazole, 1,2,4-1*H*-triazole and 4-aminotriazolium series, 4-methoxyphenacyl derivatives **3c**, **6c**, and **7c** clearly showed superior activity compared to their analogs bearing a hydrogen or chlorine atom at position 4. Furthermore, in 1,4-disubstituted triazolium series, 4-methoxy analog **8c** exhibited better profile of antifungal activity with respect to 4-chloro analog **8b**. Therefore, it can be concluded that the methoxy group of phenacyl residue improves antifungal activity. Surprisingly, 4-aminosubstitution of the triazole core (compounds **6b** and **6c**) resulted in superior activity compared to that of **7b** and **7c** against yeasts (see Table 1). Furthermore, bisphenacyl triazolium **8a** showed comparable or superior activity compared to its related azoles **5a** and **7a**. Notably, compound **8a** could be considered as a hybrid of **5a** and **7a**. Results from compounds **6** and **8** demonstrate that quaternization of the N-4 nitrogen of the phenacyl triazoles also is possible without loss of potency, and even in some cases provides an improvement of activity. Because most of antifungal azoles are too lipophilic for parenteral formulation and cannot attain reliable blood levels after administration, quaternary azolium salts such as **6b** and **6c** could be useful and promising water-soluble antifungals, although it seems that these ionic compounds maybe have a unsuitable oral bioavailability.

In conclusion, 2-hydroxyphenacyl-azole and 2-hydroxyphenacyl-azolium compounds have been identified as a new class of azole antifungal agents with a good

Table 1. Chemical structures and in vitro antifungal activity of compounds **3–8**

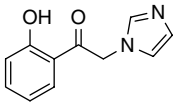
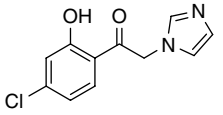
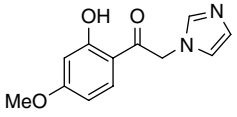
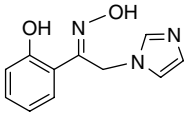
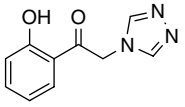
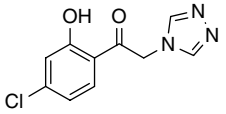
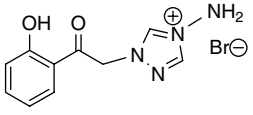
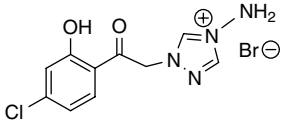
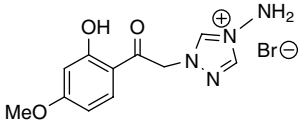
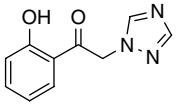
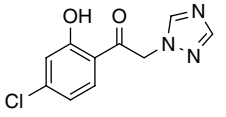
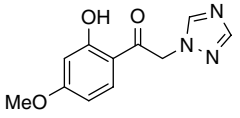
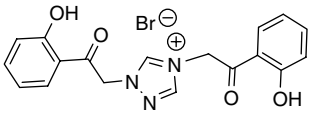
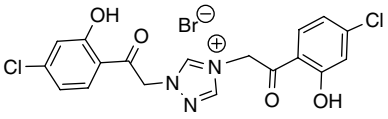
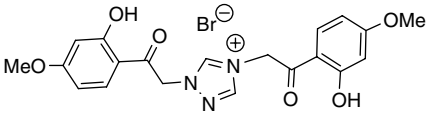
Compound	Chemical structure	Tested fungi (MICs in μg/mL)[a]			
		C. albicans	*S. cerevisiae*	*A. niger*	*M. gypseum*
3a		64	64	64	64
3b		64	0.25	64	32
3c		8	0.25	16	8
4		32	32	64	64
5a		32	16	16	16
5b		16	16	32	64
6a		64	32	32	32
6b		4	0.25	32	16
6c		2	4	16	8
7a		64	64	32	64
7b		32	64	16	16
7c		8	8	32	16

Table 1 (*continued*)

Compound	Chemical structure	Tested fungi (MICs in μg/mL)[a]			
		C. albicans	*S. cerevisiae*	*A. niger*	*M. gypseum*
8a		16	1	32	16
8b		32	8	64	32
8c		8	4	16	16
Fluconazole		8	32	32	32
Miconazole		1	1	2	8

[a] Minimum inhibitory concentrations were determined by agar dilution method for duplicate determinations.

Table 2. In vitro cytotoxic activity of selected compounds against mouse fibroblast (NIH/3T3) cell line

Compound	IC_{50} (μg/mL)[a]
3c	221 ± 11
6b	370 ± 28
6c	254 ± 12
7c	222 ± 7
8a	47 ± 5
8c	98 ± 2
Fluconazole	387 ± 17

[a] IC_{50} is the concentration required to inhibit 50% of cell growth. The values represent means ± standard deviation of triplicate determinations.

spectrum of activity. Most derivatives showed significant in vitro antifungal activities against tested fungi with low MIC values included in the range of 0.25–32 μg/mL comparable to the reference drug fluconazole. We concluded from our investigations that 2-hydroxyphenacyl-azoles (**3c** and **7c**) and 2-hydroxyphenacyl-4-aminotriazoliums (**6b** and **6c**) may be considered promising for the development of new antifungal agents with their biological activity and toxicity screening.

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13. *Typical procedure*: *the preparation of 1-(4-methoxy-2-hydroxyphenyl)-2-(1H-imidazol-1-yl)ethanone* (***3c***). A solution of 2-bromo-4′-methoxy-2′-hydroxyacetophenone **2c** (490 mg, 2.0 mmol) and imidazole (408 mg, 6.0 mmol) in DMF (4 mL) was stirred at 40 °C for 4 h. The mixture was poured into water and then extracted with $CHCl_3$. The organic phase after washing with H_2O was shaken with a solution of 10% HCl. The aqueous acid solution was neutralized with $NaHCO_3$ and the precipitate was filtered, washed with water, and dried to give **3c**. Yield 58%; mp 130–132 °C; ^{1}H NMR (80 MHz, $CDCl_3$) δ 3.87 (s, 3H, CH_3), 5.33 (s, 2H, CH_2), 6.40–6.58 (m, 3H, H-3 and H-5 Ar and H-imidazole), 7.24–7.79 (m, 3H, H-6 Ar and H-imidazole), 12.05 (br s, 1H, OH). Anal. Calcd for $C_{12}H_{12}N_2O_3$: C, 62.06; H, 5.21; N, 12.06. Found: C, 61.98; H, 5.33; N, 12.20.
14. *The preparation of 1-(2-hydroxyphenyl)-2-(1H-imidazol-1-yl)ethanone oxime* (***4a***). A solution of ketone **3a** (1.0 mmol) and hydroxylamine hydrochloride (208 mg, 3.0 mmol) in MeOH (5 mL) was stirred at room temperature for 3 d. After concentrating the reaction mixture by evaporation under reduced pressure, MeOH was replaced with water (20 mL) and neutralized with $NaHCO_3$. The precipitate was filtered by filtration, washed with water, and dried to give oxime **4a**. Yield 71%; mp 163–165 °C; ^{1}H NMR (80 MHz, DMSO-d_6) δ 5.32 (s, 2H, CH_2), 6.51–7.99 (m, 7H, phenyl and imidazole), 10.61 (s, 1H, OH), 12.04 (s, 1H, OH). Anal. Calcd for $C_{11}H_{11}N_3O_2$: C, 60.82; H, 5.10; N, 19.34. Found: C, 60.81; H, 5.28; N, 19.52.
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18. *Typical procedure: the preparation of 1-(4-chloro-2-hydroxyphenyl)-2-(4H-1,2,4-triazol-4-yl)ethanone* (***5b***). A mixture of 1*H*-1,2,4-triazole-1-propanenitrile (1.0 mmol) and 2-bromo-4′-chloro-2′-hydroxyacetophenone **2b** (1.0 mmol) in MeCN (3 mL) was refluxed with stirring for 9 h. The solvent was then evaporated and the residue was triturated with diethyl ether to give quaternary triazolium salt. This product was treated with NaOH (2.0 mmol) in H_2O (5 mL) at room temperature with stirring. After 2 d, the mixture was acidified with 10% aq HCl and neutralized with $NaHCO_3$. The precipitate was filtered, washed with water, and crystallized from methanol to afford **5b**. Yield 64%; mp 241–243 °C; IR (KBr) 3347, 3128, 1670 cm^{-1}; ^1H NMR (80 MHz, DMSO-d_6) δ 5.66 (s, 2H, CH_2), 6.80–7.15 (m, 2H, H-3 and H-5 Ar), 7.84 (d, 1H, J = 8.3 Hz, H-6 Ar), 8.43 (s, 2H, triazole), 11.57 (s, 1H, OH); Anal. Calcd for $C_{10}H_8ClN_3O_2$: C, 50.54; H, 3.39; N, 17.68. Found: C, 50.81; H, 3.30; N, 17.78.
19. *Typical procedure: the preparation of 1-(4-methoxy-2-hydroxyphenyl)-2-(4-amino-4H-1,2,4-triazoliumyl)ethanone bromide* (***6c***). A mixture of 4-amino-4*H*-1,2,4-triazole (1.51 g, 18.0 mmol) and 2-bromo-4′-methoxy-2′-hydroxyacetophenone **2c** (4.41 g, 18.0 mmol) in 2-propanol (36 mL) was refluxed for 6 h. Upon cooling the colorless salt was filtered, washed with cold 2-propanol (3× 8 mL) and diethyl ether (3× 8 mL) to give **6c**. Yield 55%; mp 177–178°C; IR (KBr) 3229, 3114, 2986, 1626, 1255, 1152 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 3.84 (s, 3H, CH_3), 6.01 (s, 2H, CH_2), 6.58 (d, 1H, J = 2.36 Hz, H-3 Ar), 6.62 (dd, 1H, J = 8.91 and 2.40 Hz, H-5 Ar), 7.17 (br s, 2H, NH_2), 7.81 (d, 1H, J = 8.90 Hz, H-6 Ar), 9.30 (s, 1H, triazole), 10.15 (s, 1H, triazole), 11.43 (s, 1H, OH). Anal. Calcd for $C_{11}H_{13}BrN_4O_3$: C, 40.14; H, 3.98; N, 17.02. Found: C, 40.32; H, 4.11; N, 16.87.
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21. *Typical procedure: the preparation of 1-(4-methoxy-2-hydroxyphenyl)-2-(1H-1,2,4-triazol-1-yl)ethanone* (***7c***). To a vigorously stirred ice-cold aqueous suspension of **6c** (8.0 mmol in 20 mL) was added concentrated HCl (1.58 g, 16.0 mmol). A solution of sodium nitrite (0.72 g, 8.4 mmol) in water (4 mL) was added dropwise at a rate to prevent excessive foaming (30 min). The mixture was permitted to come to room temperature and was stirred for another 45 min. Upon neutralization with $NaHCO_3$, the precipitate was filtered, washed with water, and crystallized from MeOH to give **7c**. Yield 97%; mp 127–129 °C; IR (KBr) 3421, 1639, 1378, 1244, 1132 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 3.84 (s, 3 H, CH_3), 5.84 (s, 2H, CH_2), 6.55 (d, 1H, J = 2.27 Hz, H-3 Ar), 6.60 (dd, 1H, J = 8.91 and 2.33 Hz, H-5 Ar), 7.87 (d, 1H, J = 8.93 Hz, H-6 Ar), 8.01 (s, 1H, triazole), 8.52 (s, 1H, triazole), 11.58 (s, 1H, OH). Anal. Calcd for $C_{11}H_{11}N_3O_3$: C, 56.65; H, 4.75; N, 18.02. Found: C, 56.76; H, 4.81; N, 17.93.
22. *Typical procedure: the preparation of 1,4-bis(4-methoxy-2-hydroxyphenacyl)-1H-1,2,4-triazolium bromide* (***8c***). To a solution of 1,2,4-triazole (69 mg, 1.0 mmol) in DMF (3 mL) was added 2-bromo-4′-methoxy-2′-hydroxyacetophenone **2c** (2.4 mmol). The mixture was stirred at room temperature for 3 d. The resulting solution was poured into ice-water (20 mL) and the precipitate was filtered, washed with water (3× 5 mL) and diethyl ether (4× 5 mL) to give **8c**. Yield 49%; mp 215–217 °C; IR (KBr) 3431, 1644, 1239, 1127 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 3.84 (s, 6H, CH_3), 6.00 (s, 2H, CH_2), 6.16 (s, 2H, CH_2), 6.59 (d, 1H, J = 2.11 Hz, H-3 Ar), 6.60 (d, 1H, J = 2.21 Hz, H-3′ Ar), 6.61–6.65 (m, 2H, H-5 Ar and H-5′ Ar), 7.84 (d, 1H, J = 8.95 Hz, H-6 Ar), 7.85 (d, 1H, J = 8.90 Hz, H-6′ Ar), 9.21 (s, 1H, triazole), 10.04 (s, 1H, triazole), 11.50 (br s, 2H, OH). Anal. Calcd for $C_{20}H_{20}BrN_3O_6$: C, 50.22; H, 4.21; N, 8.79. Found: C, 50.38; H, 4.18; N, 8.61.
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25. The in vitro cytotoxic activity of the test compounds against normal mouse fibroblast (NIH/3T3) cell line was investigated using MTT colorimetric assay. Briefly, cultures in the exponential growth phase were trypsinized and diluted in complete growth medium to give a total cell count of 5×10^4 cells/mL. One hundred microliters of suspension was added to wells of sterile 96-well plates. After plating, 50 μL of a serial dilution of every agent was added. Each compound dilution was assessed in triplicate. Three wells containing only normal mouse fibroblast (NIH/3T3) cells suspended in 150 μL of complete medium were used as control for cell viability. The plates were then incubated for 72 h. After incubation, 30 μL of a 5 mg/mL solution of MTT was added to each well and the plate was incubated for another 1 h. After incubation, the culture medium was replaced with 100 μL of DMSO. Then, the absorbance of each well was measured by using a microplate reader at 492 nm wavelengths. For each compound, dose–response curve was measured with different drug concentrations, and the concentration causing 50% cell growth inhibition (IC_{50}) compared with the control was calculated.