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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, crystal structure, and anticancer properties of cyclic monocarbonyl analogs of curcumin

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Abstract A series of novel indan-2-one and dibenzylidenepiperidin-4-one compounds were synthesized and screened for anticancer activities. The compounds are symmetrical and they have conjugated double bonds. They closely resemble the curcumin analogs which are found to possess anticancer properties. The structure of the compounds was confirmed by single crystal study and wherever the compound is a powder, the structures were confirmed by spectral data (IR, NMR, and Mass).

Keywords 2-Indanone · 1-Benzyl-4-piperidone · Aromatic aldehyde · Curcumin derivatives · Molecular docking

Introduction

Curcumin, a compound isolated form turmeric—the "Golden spice," possesses many biological properties. It is a traditional medicine for liver diseases, indigestion, rheumatic arthritis, and insect bite in India. Owing to its anticancer and antiangiogenic properties, low molecular weight and lack of toxicity, curcumin could be an ideal candidate for a chemotherapeutic agent (Adams *et al*., 2004; Aggarwal *et al*., 2003; Zeng *et al*., 2009). In the last few years, a number of natural and synthetic compounds have proven to inhibit HIV-1 IN in in vitro assays, but have failed to provide antiviral efficacy in HIV-1 infected cells. Curcumin derivatives are integrase inhibitors in vitro endowed with antiviral activity in cell-based assays (Santo *et al*., 2005). Curcumin is capable of inducing apoptosis in various cancer cell lines (Karunagaran *et al*., 2005; Lakshmi *et al*., 2008) and can suppress cancer formation (Li *et al*., 2002). Both phase I and II clinical trials show that curcumin is well tolerated and is effective for cancer patients, (Dhillon *et al*., 2006) suggesting curcumin as a potential drug in cancer prevention and therapy (Dube *et al*., 2008; Chandru *et al*., 2008). Further, it also enhances wound healing (Panchatcharam *et al*., 2006). Curcumin is nontoxic even at high dosage. It has been classified as "generally recognized as safe" (GRAS) by the National Cancer Institute (Osawa *et al*., 1995).

The Curcumin analogs (1,5-diaryl-3-oxo-1,4-pentadienyl group) have been mounted on a variety of cyclic scaffolds leading to the discovery of a number of potent cytotoxins (Pati *et al*., 2009; Fadda *et al*., 2009). This group is considered to react at a primary binding site (Fig. 1a). However, the magnitude of the bioactivity observed will be influenced by the presence of other structural units in the molecule, which align at an auxiliary site (Fig. 1b).

Several studies have revealed that compounds in which R is an acyl group possess increased cytotoxic potencies compared to the analogs when R is a hydrogen atom (Das *et al*., 2007). Thus, by expanding the size of the molecules, there is a possibility of additional binding of the ligand at a receptor which results in the lowering of the IC_{50} values (Pati *et al*., 2009). In curcumin analogs, the substitution pattern of the aromatic moiety is of importance. The most active compounds have a fluorine atom or a methoxyl

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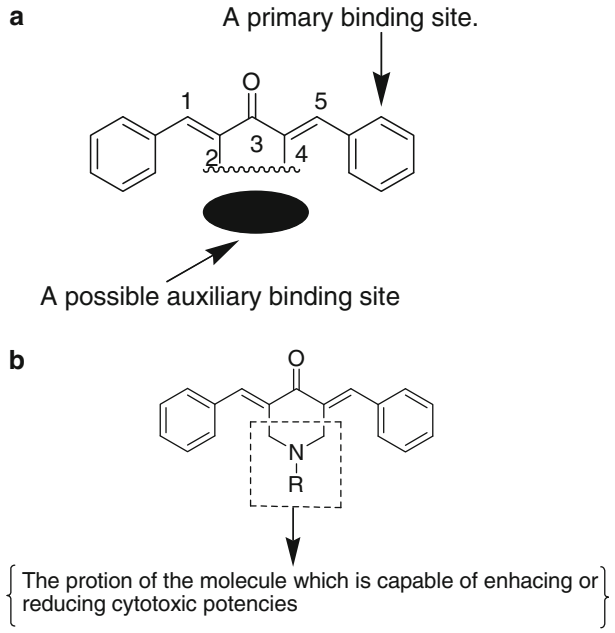


Fig. 1 **a** Primary binding sites. **b** A possible auxiliary binding site

group in the *ortho* position of the OH-group of the *p*-hydroxyphenyl moiety (Gafner *et al*., 2004). *Ortho*-substituents consistently exhibit better activity than their *meta*- and *para*-counterparts (Adams *et al*., 2004).

Experimental

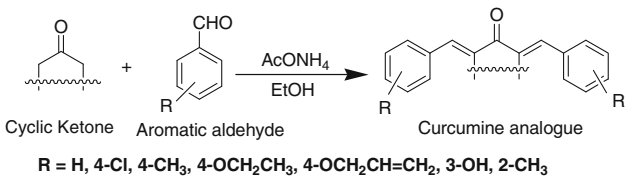
Chemistry

The synthesis and biological evaluation of curcumin analogs have been reported previously. In this study, the authors wish to report a new synthetic method (Scheme 1), using ammonium acetate, for monocarbonyl curcumin analogs (symmetrical aromatic moieties) with more stable chemical structures, as well as their anticancer screening in various cell lines.

This procedure is more convenient than the literature method (Adams *et al*., 2004; Pati *et al*., 2008, 2009; Liang *et al*., 2008a), easy to synthesis, isolate, and less expensive. A mixture of cyclic ketone (0.01 mol) and the appropriate aldehyde (0.02 mol) in alcoholic ammonium acetate (15 ml, 0.01 mol) was gently heated and left overnight at room temperature. The separated solid was purified by column chromatography.

Biology

The two series (IND—Fig. 2 and BP—Fig. 3) of eight monocarbonyl curcumin analogs were synthesized and screened for anticancer activity. Recently, antibacterial activities of monocarbonyl analogs of curcumin have also been discussed (Liang *et al*., 2008b). To the best of our knowledge, this is the first time an investigation has been made for the anticancer activity of IND series (Fig. 2). The anticancer screening was performed using cell lines, HCT-116 (Colon cancer), Panc-1 (Pancreatic cancer), H460 (Non small lung cancer), Calu-1 (Lung cancer), ACHN (Renal cancer). The results compare well with W138 (Normal lung fibroblast cells).



Scheme 1 General synthesis of curcumin analogs (**1–8**)

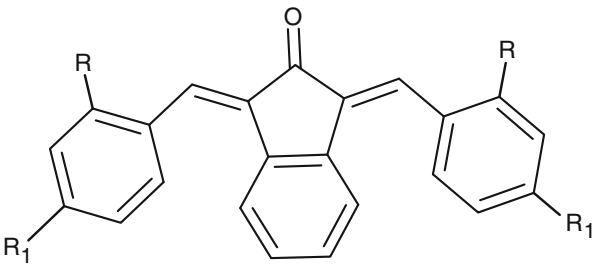
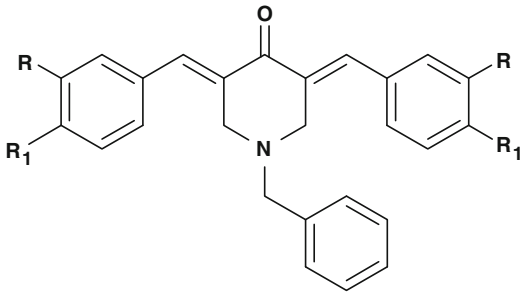


Fig. 2 Compound **1–3**

Fig. 3 Compound **4–8**



1. (PCIND);R = H, R_1 = Cl
2. (PMIND);R = H, R_1 = OCH_3
3. (OMIND);R = OCH_3, R_1 = H
4. (BP);R = H, R_1 = H
5. (PMBP);R = H, R_1 = OCH_3
6. (PEBP);R = H, R_1 = OCH_2CH_3
7. (PABP);R=H,R_1 =$OCH_2CH=CH_2$
8. (MHBP); R = OH, R_1 = H

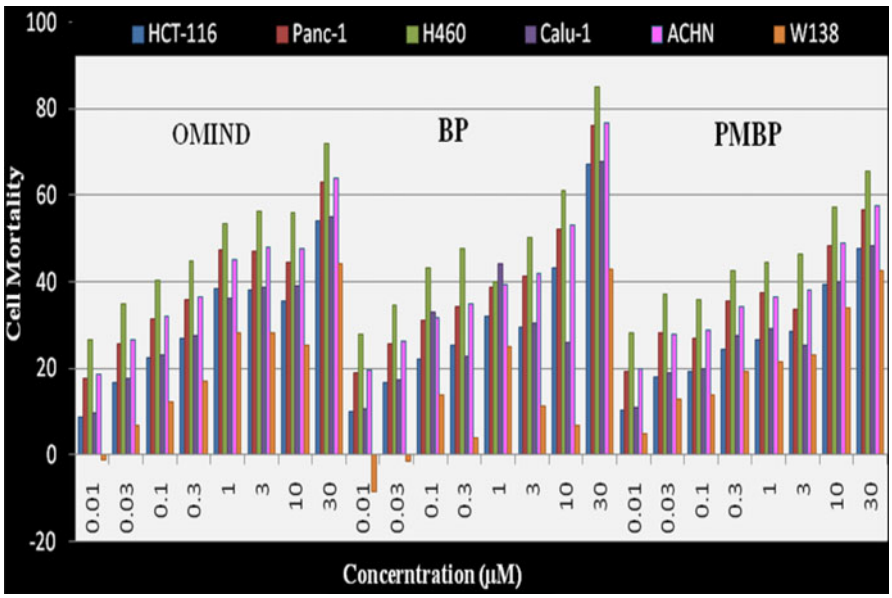


Chart 1 Cell mortality for OMIND, BP, and PMBP in various cell lines at different concentration (μM)

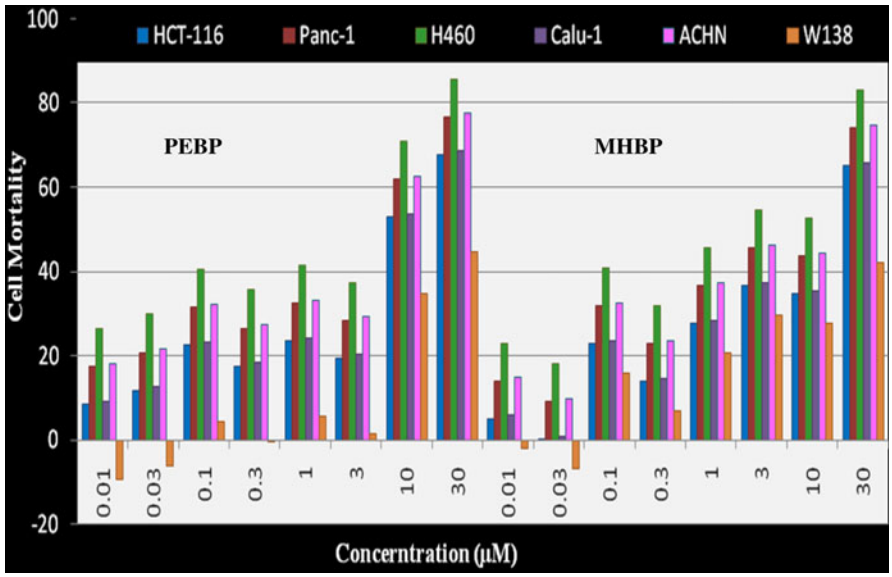


Chart 2 Cell mortality for PEBP and MHBP in various cell lines at different concentration (μM)

Results and discussion

Chemistry

The structures of curcumin analogs (**1–8**) were in accordance with their crystal data and spectroscopic data. The FT-IR spectrum of the compounds in general exhibited the absorption band at around 1705–1569 cm^{-1} indicating the carbonyl group and olefinic bonds of α,α′-(EE)-bis(substituted benzylidene)cycloalkanones are in conjugation. The mass spectrum of the compounds showed its respective peaks. In the ^{1}H-NMR spectrum of the compounds, the vinylic protons either occur as singlet at around δ 7.61–7.49 ppm or as merged ones with the multiplet of aromatic protons ranging from δ 8.25 to 6.96 ppm.

Biology

The CCK-8 nonradioactive colorimetric assay was carried out (Jing-yan *et al*., 2006; Cell Counting Kit-8 technical manual) to characterize the in vitro growth of human tumor cell lines as well as to test the cytotoxic activity of these compounds. The compound PEBP showed the highest anticancer activity among the compounds tested, and it showed IC_{50} of 8.3 μM in Colon cancer, 7.2 μM in Pancreatic cancer, 5.5 μM for Non small lung cancer, 8.1 μM for Lung cancer, and 6.9 μM for Renal cancer cells (Chart 2). The activity was tested in the concentration ranges of 0.01–30 μM. The activity was found to increase with increase in concentration. Fortunately, none of the compounds is found to be toxic in normal primary lung

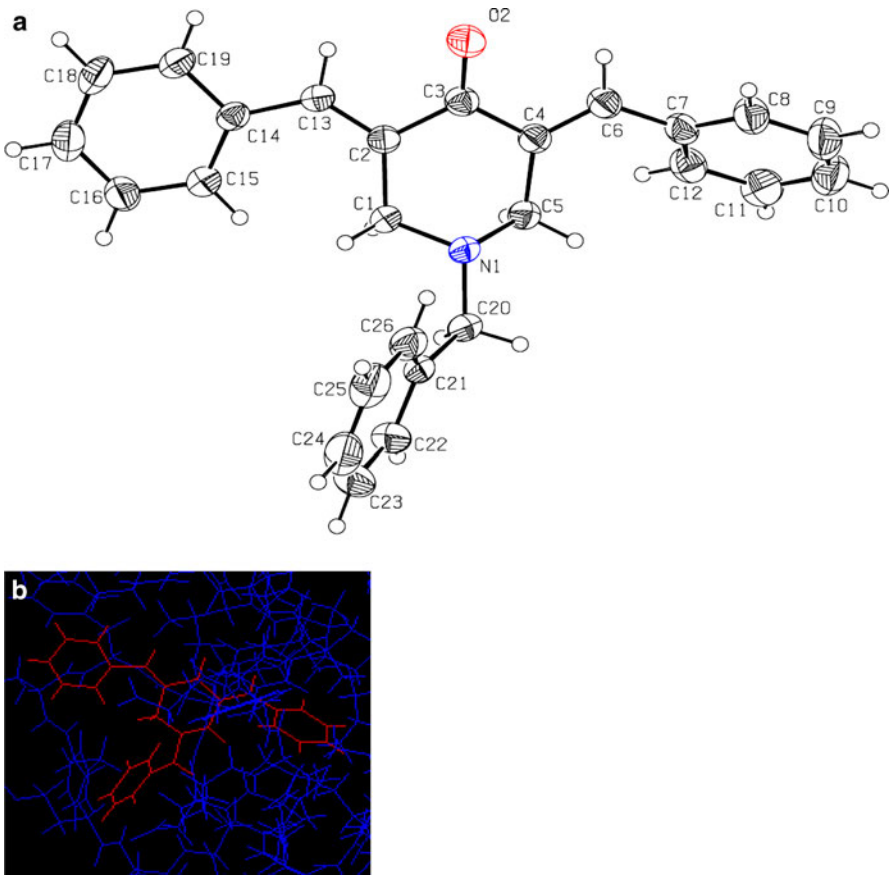


Fig. 4 **a** ORTEP diagram of BP. **b** Docking of receptor model—1m3g with BP

fibroblast cells. If we see the order of activity, the order is roughly as follows OMIND < PMBP < BP < MHBP < PEBP (Charts 1, 2). Out of the two series, BP derivatives (Fig. 3) are more active than IND series (Fig. 2). This may be due to the reason that the auxiliary binding site in BP is capable of enhancing the activity. However, in the case of IND series, the auxiliary binding site is not that capable of increasing the activity. This is clear from the Fig. 1a and b. This gets support from the earlier observation in which some BP compounds were reported for selective toxicity for malignant cells (Pati *et al*., 2008). However, the activity of BP series is moderately affected by the substitution present in it. Within the BP series, the highest activity is shown by PEBP followed by MHBP, BP, and PMBP (Charts 1, 2). This order may be due to the reason that, out of ethoxy and methoxy, ethoxy is more active than the methoxy due to the large chain of ethyl group. The moderately good activity of MHBP is due to the reason that OH derivatives normally increase the anticancer activity.

In the case of Colon cancer, the order follows OMIND < MHBP < BP < PEBP < PMBP. Here, again the ethyl group in the substitution plays a major role. With Pancreatic cancer, the order follows OMIND < MHBP < PMBP < BP < PEBP. Here also the ethyl substitution is responsible for the highest activity. However, in the case of Non small lung cancer, the order of the activity is changed completely, and here, the most active one is OMIND and the least active is PEBP. The order follows PEBP < PMBP < BP < MHBP < OMIND. Here, the activity of all the compounds increases tremendously, when compared to other cancers. In the case of Lung cancer, again the order of activity resembles the order followed in Colon cancer PMBP < OMIND < MHBP ≈ BP < PEBP. The activity here is not as pronounced as in the case of Non small lung cancer. In the case of Renal cancer, the activity follows the order PMBP < OMIND < MHBP < BP < PEBP.

From the above observations, the PEBP (Chart 2) is found to be more active than all other compounds.

Molecular modeling

Before the compounds were tested in in vitro, molecular modeling studies were performed. Docking studies were undertaken by means of the patch dock program (Patch-Dock Server 2008), with the aim of better understanding the binding mode of the curcumin analogs to the biological target. We got crystalline structure for only two compounds, namely, BP (Fig. 4a) and PMBP (Fig. 5a), and these two were subjected to docking studies.

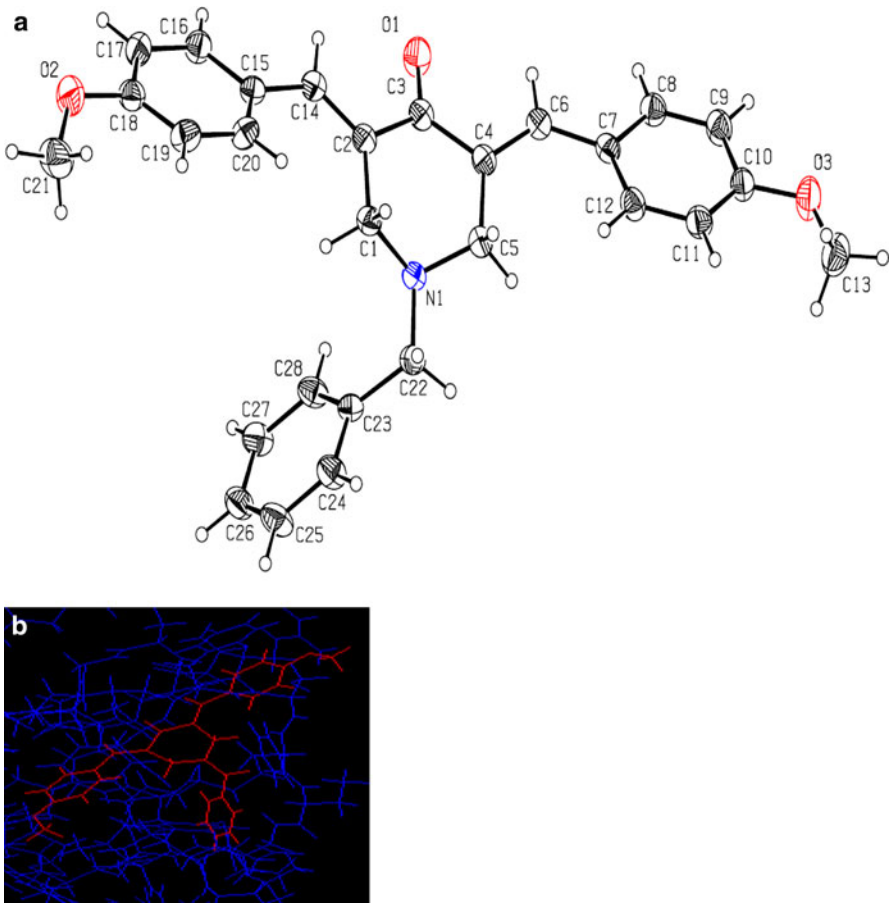


Fig. 5 **a** ORTEP diagram of PM. **b** Docking of receptor model—1m3g with PMBP

Tyrosine phosphorylation of proteins is required for signal transduction in cells and for growth regulation. A mitogen-induced gene (PAC-1) has been cloned from human T cells and encodes a 32-kDa protein that contains a sequence that defines the enzymatic site of known protein phosphotyrosine phosphatases (PTPases). We subjected these two compounds for molecular modeling studies against catalytic domain of MAPK protein phosphatase (PAC-1) (residue 170–314) (Wu *et al*., 2007; RCSB Protein Data Bank—Receptor 1m3g).

Initial data reveal that the compound binds to PAC-1 and modulates its expression (Figs. 4b, 5b). The PAC-1 is a direct target of E2F-1, which plays a main role in cell cycle regulation. The enhanced activity of this compound in cancer cells may be due to PAC-1 modulation in cancer cells.

Conclusion

The above discussion reveals that compounds in the BP (Fig. 2) and IND series (Fig. 3) have anticancer properties. These heterocycles could be considered as useful templates for future development and further derivatization to obtain more potent and selective anticancer agents. Substantial structural modifications of curcumin have an important potential for drug development.

Spectral data

1,3-Bis(4-chlorobenzylidene)-indan-2-one (PCIND)

Yellow amorphous powder; mp 182°C; 83% Yield; IR (KBr) cm^{-1} 2918, 1713, 1623, 776; ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 7.27–7.74 (14H, olefinic protons and ArH); GC–MS: $m/z = 376$ $(M - 1)^{+}$.

1,3-Bis(4-methoxybenzylidene)-indan-2-one (PMIND)

Yellow crystal; mp 156–160°C; 72% Yield; IR (KBr) cm^{-1}: 2958, 2838, 1705, 1593; ^{1}H-NMR ($CDCl_3$, 300 MHz): $\delta = 3.89$ (6H, s, OCH_3), 6.96–8.29 (14H, olefinic protons and ArH); ^{13}C NMR ($CDCl_3$, 500 MHz): $\delta = 55.49, 113.71, 114.07, 114.12, 119.52, 123.10, 123.16, 127.65, 127.74, 127.87, 128.60, 129.07, 130.05, 131.04, 131.09, 133.04, 133.31, 133.71, 134.03, 134.84, 140.82, 160.44, 160.60, 161.38, 192.76$. LC–MS: $m/z = 369$ $(M + 1)^{+}$.

1,3-Bis(2-methoxybenzylidene)-indan-2-one (OMIND)

Yellow amorphous powder; mp 108–112°C; 78% Yield; IR (KBr) cm^{-1} 3070, 2933, 2836, 1708,1596; ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 3.84 (6H, s OCH_3), 6.98–7.80 (14H, olefinic protons and ArH); ^{13}C NMR ($CDCl_3$, 500 MHz): δ = 55.58, 55.69, 110.24, 110.96, 111.01, 120.23, 123.48, 124.37, 128.56, 129.46, 129.58, 130.99, 158.13, 158.16, 194.41. GC–MS: m/z = 368 (M^+).

1-Benzyl-3,5-dibenzylidenepiperidin-4-one (BP)

Yellow block Crystal; mp 152–154°C; 97% Yield; IR (KBr) cm^{-1} 3061, 3019, 2798,2755, 1671,1569; ^{1}H-NMR (DMSO-d_6, 300 MHz): δ 3.69 (2H, s), 3.81 (4H, s), 7.18–7.42 (15H, ArH), 7.61 (2H, s, olefinic protons); GC–MS: m/z = 365 (M^+).

3,5-Bis(4-methoxybenzylidene)-1-benzylpiperidin-4-one (PMBP)

Yellow block Crystal; mp 150–154°C; 83% Yield; IR (KBr) cm^{-1} 3063, 2935, 2836, 2743, 1668, 1577; ^{1}H-NMR (DMSO-d_6, 300 MHz): δ 3.71 (2H, s), 3.78 (6H, s, OCH_3), 3.85 (4H, s) 6.97–7.89 (15H, olefinic protons and ArH); LC–MS: m/z = 426 $(M + 1)^+$.

3,5-Bis(4-ethoxybenzylidene)-1-benzylpiperidin-4-one (PEBP)

Yellow needle Crystal; mp 110–114°C; 81% Yield; IR (KBr) cm^{-1} 3062, 3031, 2764,1670, 1577; ^{1}H-NMR (DMSO-d_6, 300 MHz): δ 1.29–1.33 (6H, t, CH_2CH_3) 3.70 (2H, s), 3.77 (4H, s), 4.01–4.08 (4H, q, OCH_2) 6.94–7.39 (13H, ArH) 7.54 (2H, s, olefinic protons); LC–MS: m/z = 454 $(M + 1)^+$.

3,5-Bis(4-allyloxybenzylidene)-1-benzylpiperidin-4-one (PABP)

Yellow Crystal; mp 114–116°C; 91% Yield; IR (KBr) cm^{-1} 3063, 3028, 2887, 2743,1670, 1600, 1577; ^{1}H-NMR (DMSO-d_6, 300 MHz): δ 3.70 (2H, s), 3.78 (4H, s), 4.59–4.61 (4H, d, OCH_2) 5.24–5.41 (4H, CH_2), 5.98–6.04 (2H, CH), 6.94–7.40 (13H, ArH) 7.54 (2H, s, olefinic protons); MS: m/z = 478 $(M + 1)^+$.

3,5-Bis(3-hydroxybenzylidene)-1-benzylpiperidin-4-one (MHBP)

Pale Yellow Crystal; mp 180–184°C; 83% Yield; IR (KBr) cm^{-1} 3299, 2772,1666; ^{1}H-NMR (DMSO-d_6, 300 MHz): δ 3.69 (2H, s), 3.78 (4H, s), 6.76–7.26 (13H, ArH) 7.49 (2H, s, olefinic protons), 9.55 (2H, OH); LC–MS: m/z = 398 $(M + 1)^+$.

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