Med Chem Res
DOI 10.1007/s00044-014-1008-y

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

# Chemoselective *N*-benzylation of 2-thiohydantoins and 2-thiobarbituric acids catalyzed by PEG-stabilized Ni nanoparticles and their anti-microbial activities

**Sneha · Jitender M. Khurana · Chetan Sharma · K. R. Aneja**

Received: 29 November 2012 / Accepted: 14 April 2014


**Abstract** An efficient one pot chemoselective *N*-benzylation of 2-thiohydantoins and 2-thiobarbituric acids catalyzed by PEG-stabilized Ni nanoparticles has been reported, wherein the NH group of thiohydantoins and thiobarbituric acids is selectively benzylated and no *S*-benzylation is observed. Mildness and environment friendly approach of the protocol gives it an edge over other conventional methods. These compounds were tested in vitro for their antibacterial activity against Gram-positive bacteria namely, *Staphylococcus aureus* and *Bacillus subtilis* and their inhibitory action against two strains of fungus.

**Keywords** Ni nanoparticles · *N*-Benzylation · 2-Thiohydantoin · 2-Thiobarbituric acid · Anti-microbial activity

## Introduction

Thiohydantoins and their derivatives represent an important class of biologically active molecules having broad medicinal applications viz. anticancer (Shih *et al*., 2004; Takahashi *et al*., 2005), anticonvulsant (Chui *et al*., 2004), anti-microbial (Gulati *et al*., 1994), hypolipidemic (Tompkin, 1986), hypotensive (Gadwood *et al*., 1993), and agrochemical (herbicidal and fungicidal) (Mizuno *et al*., 2000) applications. Furthermore, many thiohydantoins are responsible for inhibition of fatty acid hydrolases (Muccioli *et al*., 2006), glycogen phosphorylases (Agasimundin *et al*., 1998), amylases (Kandra *et al*., 2005), and serine proteases (He *et al*., 2000). 5-Arylidene-2-thiohydantoins are reported to possess anti-mycobacterial and anti-microbial activities (Szymańska *et al*., 2002). Recent studies have shown that some arylidene(thio)hydantoin derivatives possess P-gp modulating properties in cancer cells in the range of verapamil or higher (Spengler *et al*., 2010). Handzlik and co-workers have synthesized a series of new 5-arylidene(thio)hydantoin derivatives as modulators of cancer efflux pump (Handzlik *et al*., 2012). Thiohydantoin derivatives have been designed and synthesized for detecting tau pathology in the brains of patients with Alzheimer's disease (Aly *et al*., 2004). 5-Benzylidene-2-thiohydantoins have been reported to undergo Mannich reaction with formaldehyde and morpholine to give the corresponding Mannich products (Ono *et al*., 2011).

Barbituric acid derivatives namely, arylidene barbituric acids as well as their 2-thio analogs are useful as intermediates in synthesis of heterocyclic compounds (Bojarski *et al*., 1985), benzyl barbituric derivatives (Frangin *et al*., 1986), oxadiazaflavines (Figueroa-Villar *et al*., 1992), and unsymmetrical disulfides (Tanaka *et al*., 1988). 5-Benzylidene thiobarbiturate derivatives have been evaluated as tyrosinase inhibitors and antibacterial agents (Yan *et al*., 2009).

In recent times, there has been budding interest in using nickel nanoparticles in organic synthesis because of their potent catalytic activity, easy preparation and high stability. Ni nanoparticles overcome the shortcoming of recyclability of homogeneous nickel catalysts thereby behaving as an efficient heterogeneous catalyst. In continuation to our ongoing interest (Khurana and Vij, 2010, 2011, Khurana *et al*., 2012) in identifying newer applications of

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Published online: 20 May 2014

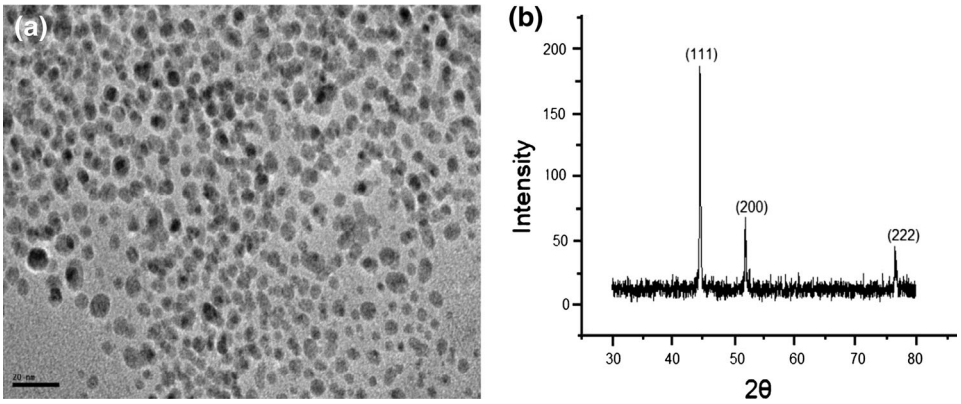



**Fig. 1** **a** TEM image of PEG-stabilized Ni nanoparticles. The *scale bar* corresponds to 20 nm in the image; **b** X-ray diffraction pattern of PEG-Ni nanoparticles

nickel nanoparticles, we have investigated a new procedure for *N*-benzylation of 2-thiohydantoin and 2-thiobarbituric acid derivatives in presence of Ni nanoparticles as active catalysts.

## Results and discussion

We report in this paper, a novel protocol for the synthesis of new *N*-benzylated arylidene thiohydantoins viz. 3-benzyl-5-arylidene-2-thioxoimidazolidin-4-ones (IIIa-h) and *N*-benzylated arylidene thiobarbiturates viz. 1,3-dibenzyl-5-arylidene-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-diones (IVa-i) in ethylene glycol using PEG-stabilized Ni nanoparticles as semi-heterogeneous catalysts. Monodispersed Ni nanoparticles were synthesized from $NiCl_2.6H_2O$ as metal salt precursor and $NaBH_4$ as reducing agent using polyethylene glycol (PEG-4000) as stabilizing agent. The average size of metal nanoparticles was found to be 7 nm through TEM (Fig. 1a) and XRD analysis (Fig. 1b). XRD pattern confirmed the metallic nature of Ni nanoparticles. The X-ray diffraction shows peaks at $2\theta = 44.5^{o}$, $51.8^{o}$, and $76.4^{o}$, corresponding to the (111), (200), and (222) lattice planes which clearly indicate the presence of pure elemental Ni with FCC structure. The size from the XRD data was calculated to be 6.9 nm and is consistent with TEM results.

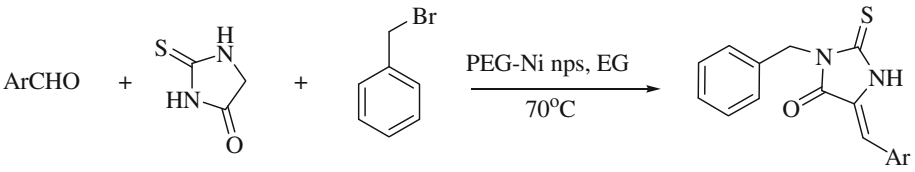
The formation of *N*-benzylated arylidene thiohydantoins can be achieved in two steps either by benzylation of thiohydantoins followed by Knoevenagel condensation with aldehydes or by Knoevenagel condensation of thiohydantoin with aldehydes followed by benzylation. We followed the former approach. Therefore, *N*-benzylation of 2-thiohydantoin was attempted in PEG-Ni nanoparticles dispersion in ethylene glycol. The reaction of 2-thiohydantoin (1.0 equiv.) and benzyl bromide (2.0 equiv.) was carried out in Ni nanoparticles dispersion in ethylene glycol (2.0 mL/0.1 g of substrate) at different temperatures. There was no reaction at room temperature while reaction at higher temperatures gave a mixture of products instead of the desired product. We speculated this to be because of the unprotected *C*-5 position. We thought that second approach of Knoevenagel condensation of thiohydantoin with aryl aldehyde followed by benzylation could be productive. Alternatively, we thought a one pot reaction of the three components could be attempted. Therefore, we attempted a one pot Knoevenagel condensation and benzylation. The reaction between 4-nitrobenzaldehyde (1.0 equiv.), 2-thiohydantoin (1.0 equiv.), and benzyl bromide (2.0 equiv.) in Ni nanoparticles dispersion in ethylene glycol (2 mL/0.1 g aldehyde) at room temperature showed the formation of a new product which was believed to be 5-(4-nitrobenzylidene)-2-thioxo-imidazolidin-4-one, the Knoevenagel condensation product rather than the desired product, as identified by co-TLC with authentic sample. It is likely that the reaction came to a halt after first step. The above reaction was then repeated at 50 °C under otherwise identical conditions. A new spot was observed as monitored by TLC using petroleum ether: ethyl acetate (60:40, v/v) as eluent which did not correspond to the Knoevenagel condensation product. However, the reaction was incomplete even after long time of stirring (6 h). Encouraged by the result, the above reaction was repeated at still higher temperature of 70 °C. The reaction was complete after 30 min, and after work-up, 3-benzyl-5-(4-nitrobenzylidene)-2-thioxoimidazolidin-4-one (IIIa) was isolated in 90 % yield. The benzylation took place exclusively at *N*-3 and the *N*-1 position remained intact. Also, no *S*-alkylated product was obtained.

Attempts to obtain 1,3-dibenzyl derivative of thiohydantoin by varying the reaction conditions (elevated temperatures and higher catalyst loading) were unsuccessful. The reaction of the pre-formed Knoevenagel product, 5-(4-nitrobenzylidene)-2-thioxoimidazolidin-4-one, with double molar ratio of benzyl bromide also resulted in the formation of **IIIa** only instead of the dibenzyl product. Since the dibenzylated product could not be obtained, a reaction was attempted with equivalent amounts of 4-nitrobenzaldehyde,

2-thiohydantoin, and benzyl bromide at 70 °C in presence of PEG-Ni nanoparticles dispersion in ethylene glycol (2 mL/0.1 g of Ia). It was observed that the reaction was complete in 30 min with 91 % yield of **IIIa**. These results are listed in Table 1.

The scope of the reaction was extended to different aldehydes with equivalent amounts of 2-thiohydantoin and benzyl bromide under otherwise similar conditions (Eq. 1). The corresponding novel products (IIIb-h) were obtained in high yields. The results are summarized in Table 2.

In order to authenticate the effective involvement of Ni nanoparticles in the reaction, a blank reaction was carried out with **Ia**, **IIa**, and benzyl bromide in ethylene glycol in the absence of Ni nanoparticles at 70 °C. The reaction was incomplete even after 7 h, and the product isolated was identified to be Knoevenagel product 5-(4-nitrobenzylidene)-2-thioxoimidazolidin-4-one and not the desired product **IIIa**. In another experiment, Ni nanoparticles were isolated from the sample, followed by washing several times with absolute ethanol and subsequently redispersing




I IIa IIIa-h 1

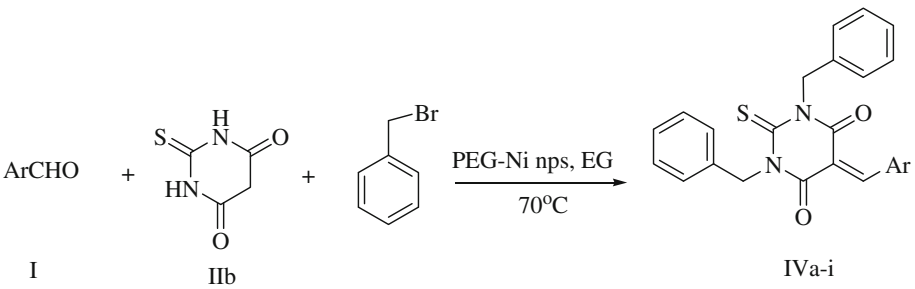
Encouraged by the results obtained with 2-thiohydantoin, we decided to extend the protocol with 2-thiobarbituric acid. A reaction of 4-nitrobenzaldehyde (1.0 equiv.), 2-thiobarbituric acid (1.0 equiv.), and benzyl bromide (2.0 equiv.) was attempted in Ni nanoparticles dispersion in ethylene glycol (2.0 mL/0.1 g Ia) at room temperature. No reaction occurred since thiobarbituric acid has low solubility in ethylene glycol at room temperature. Considering this, the reaction was carried out at 70 °C. The reaction was found to be complete in 45 min as monitored by TLC using petroleum ether: ethyl acetate (60:40, v/v) as eluent. The product obtained after work-up was identified to be 1,3-dibenzyl-5-(4-nitrobenzylidene)-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione (92 %). The benzylation at *N*-1 and *N*-3 positions is equally probable in 2-thiobarbituric acid unlike in 2-thiohydantoin where *N*-3 position is more reactive due to its favorable location between two activating groups. A variety of aryl aldehydes were screened to establish the generality of the reaction. All the reactions proceeded smoothly, and the desired products were obtained in high yields (IVa-i) (Eq. 2, Table 3).

them in ethylene glycol. The dispersion so obtained was then used for the reaction of **Ia**, **IIa**, and benzyl bromide. The reaction was complete within 35 min and 90 % of **IIIa** was isolated.

Plausible mechanism for the formation of 3-benzyl-5-benzylidene-2-thiohydantoin is outlined in Scheme 1.

The intermediate formation of 5-arylidene-2-thiohydantoin has been confirmed by an independent reaction. We carried out the synthesis of **IIIa** in two steps. First, the Knoevenagel product 5-(4-nitrobenzylidene)-2-thioxoimidazolidin-4-one was prepared by the condensation of 4-nitrobenzaldehyde (Ia) and 2-thiohydantoin in presence of PEG-stabilized Ni nanoparticles at room temperature. It was then treated with benzyl bromide in presence of PEG-stabilized Ni nanoparticles dispersion in ethylene glycol at 70 °C to give the product **IIIa** (88 %) in 35 min. This supports the proposed pathway for the intermediate 5-arylidene-2-thiohydantoins.

The recyclability of Ni nanoparticles in these reactions was also investigated. The catalyst could be recycled. The product was separated from the reaction mixture by




I IIb IVa-i 2

**Table 1** Reaction of 4-nitrobenzaldehyde (Ia, 1.0 equiv.), 2-thiohydantoin (IIa, 1.0 equiv.), and benzyl bromide (2.0 equiv.) in presence of PEG-Ni nanoparticles

| Entry | Volume of Ni nps dispersion (mL)/0.1 g of Ia | wt% (Ni: 0.1 g of Ia) | Temp. (°C) | Time (h) | (%) Yield |
|---|---|---|---|---|---|
| 1. | 2.0 | 0.0235 | r.t. | 3 | 85[a] |
| 2. | 2.0 | 0.0235 | 50 | 4 | 60[b] |
| 3. | 2.0 | 0.0235 | 70 | 0.5 | 90[c] |
| 4. | 2.0 | 0.0235 | 100 | 0.5 | 91[c] |
| 5. | 2.0 | 0.0235 | 140 | 0.5 | 90[c] |
| 6. | 4.0 | 0.0470 | 70 | 0.5 | 86[c] |
| 7. | 8.0 | 0.0940 | 70 | 0.5 | 82[c] |
| 8. | 2.0 | 0.0235 | 70 | 0.5 | 91[c,d] |

*r.t.* room temperature

[a] Knoevenagel product, 5-(4-nitro-benzylidene)-2-thioxo-imidazolidin-4-one, was isolated

[b] Incomplete reaction

[c] 3-Benzyl-5-(4-nitrobenzylidene)-2-thioxoimidazolidin-4-one (IIIa) was isolated

[d] Reaction carried out with 4-nitrobenzaldehyde (1.0 equiv.), 2-thiohydantoin (1.0 equiv.) and benzyl bromide (1.0 equiv.)

**Table 2** Ni nanoparticles catalyzed synthesis of 3-benzyl-5-arylidene-2-thioxoimidazolidin-4-ones (**III**)

| Entry | Ar (ArCHO) | Product | Time (min) | (%) Yield |
|---|---|---|---|---|
| 1. | 2,4-$Cl_2C_6H_3$ (Ib) | **IIIb** | 50 | 91 |
| 2. | 3,4-$(MeO)_2C_6H_3$ (Ic) | **IIIc** | 30 | 93 |
| 3. | 4-$FC_6H_4$ (Id) | **IIId** | 45 | 90 |
| 4. | 3-$O_2NC_6H_4$ (Ie) | **IIIe** | 40 | 89 |
| 5. | 4-$(NC)C_6H_4$ (If) | **IIIf** | 30 | 94 |
| 6. | 3-$(NC)C_6H_4$ (Ig) | **IIIg** | 30 | 93 |
| 7. | 4-$(CH_3)_2NC_6H_4$ (Ih) | **IIIh** | 60 | 92 |

Reaction carried out with aldehyde (1.0 equiv.), 2-thiohydantoin (1.0 equiv.), and benzyl bromide (1.0 equiv.) at 70 °C in presence of PEG-Ni nps in ethylene glycol (2.0 mL/0.1 g of I)

extraction with ethyl acetate several times as it was completely immiscible with ethylene glycol at room temperature. The recovered ethylene glycol layer containing the Ni nanoparticles could be reused for the subsequent cycles after sonication. It was found that the catalyst retained optimum activity till four cycles (IIIa, 90–84 %) after which a drop in yield (76 %) was observed.

## Biological screening

A total of 16 chemical compounds were screened for their antibacterial and antifungal activity. The compounds possessed variable antibacterial activity against Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*) bacteria and antifungal activity against *Aspergillus niger* and *Aspergillus flavus*. However, these compounds did not exhibit any activity against Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria. Positive controls produced significantly sized inhibition zones against the tested bacteria and fungi, however, negative control produced no observable inhibitory effect against any of the test organism as shown in Table 4 and Table 6.

**Table 3** Ni nanoparticles catalyzed synthesis of 1,3-dibenzyl-5-arylidene-2-thioxodihydro pyrimidine-4,6(1*H*,5*H*)diones (**IV**)

| Entry | Ar (ArCHO) | Product | Time (min) | (%) Yield |
|---|---|---|---|---|
| 1. | 4-$O_2NC_6H_4$ (Ia) | **IVa** | 45 | 92 |
| 2. | 4-$FC_6H_4$ (Id) | **IVb** | 75 | 88 |
| 3. | 3-$O_2NC_6H_4$ (Ie) | **IVc** | 40 | 90 |
| 4. | 2,5-$(OMe)_2C_6H_3$ (Ii) | **IVd** | 60 | 89 |
| 5. | 4-$(Me)_2CHC_6H_4$ (Ij) | **IVe** | 50 | 89 |
| 6. | 2-$MeC_6H_4$ (Ik) | **IVf** | 60 | 91 |
| 7. | 2-$O_2NC_6H_4$ (Il) | **IVg** | 45 | 90 |
| 8. | 2-Naphthyl (Im) | **IVh** | 60 | 87 |
| 9. | 3-$ClC_6H_4$ (In) | **IVi** | 35 | 92 |

Reaction carried out with aldehyde (1.0 equiv.), 2-thiobarbituric acid (1.0 equiv.) and benzyl bromide (2.0 equiv.) at 70 °C in presence of PEG-Ni nps in ethylene glycol (2.0 mL/0.1 g of I)

The tested compounds showed zone of inhibition ranging between 13.6 mm and 21.3 mm against the Gram-positive bacteria. On the basis of zone of inhibition produced against the test bacterium, compound **IVe** was found to be most effective against *B.subtilis* and *S. aureus*, with zone of inhibition of 19.6 mm and 21.3 mm, respectively (Table 4; Fig. 2). In the whole series, the MIC of chemical compounds ranged between 32 and 512 µg/mL against Gram-positive bacteria. Compound **IVe** was found to be best as it exhibited the lowest MIC of 32 µg/mL against *B.subtilis* and two compounds namely, **IVe** and **IVg** showed lowest MIC of 64 µg/mL against *S. aureus* (Table 5; Fig. 3).

All the compounds were screened for their antifungal activity also. Two compounds, **IIIe** and **IVe**, showed more than 50 % inhibition of mycelial growth against *A. flavus* whereas only one compound **IVe** showed more than 50 % inhibition of mycelial growth against *A. niger* (Table 6; Fig. 4).

Among all the compounds, compound **IVe** showed good activity against the Gram-positive bacteria and tested fungal pathogens. Therefore, this compound can be further explored in the pharmaceutical industry, after testing its toxicity to human beings.

In conclusion, we have reported a mild and highly chemoselective *N*-benzylation of 2-thiohydantoins and

**Scheme 1** Proposed pathway for the synthesis of 3-benzyl-5-arylidene-2-thiohydantoin

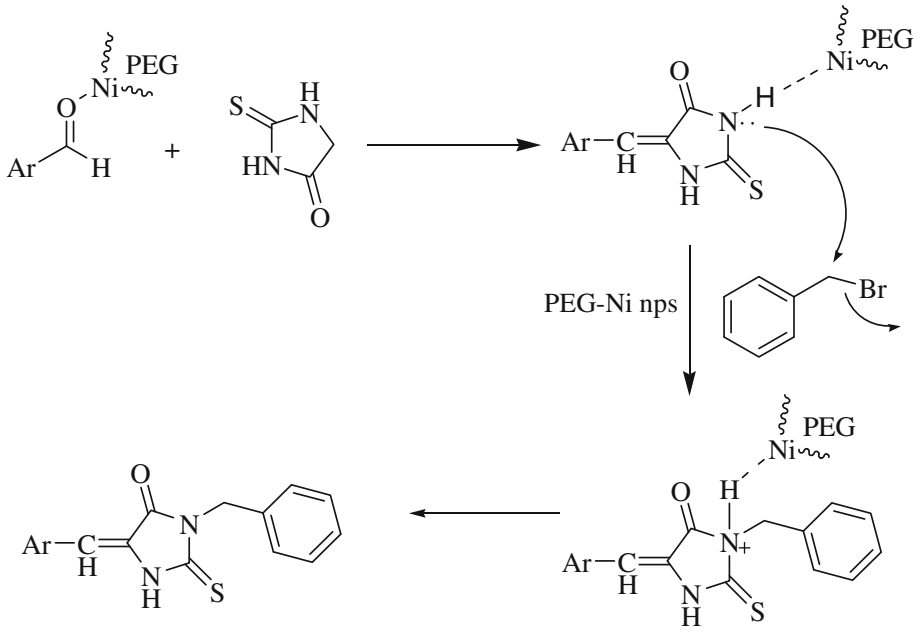



**Table 4** Antibacterial activity of compounds III and IV through agar well diffusion method

| Compounds | Diameter of growth of inhibition zone (mm)[a] | | | |
|---|---|---|---|---|
| | *Staphylococcus aureus* | *Bacillus subtilis* | *Escherichia coli* | *Pseudomonas aeruginosa* |
| **IIIa** | 13.6 | 15.3 | – | – |
| **IIIb** | 13.3 | 15.6 | – | – |
| **IIId** | 15.6 | 17.3 | – | – |
| **IIIe** | 14.3 | 15.6 | **–** | **–** |
| **IIIf** | 14.6 | 14.3 | – | – |
| **IIIg** | 16.3 | 18.0 | | |
| **IIIh** | 14.3 | 15.6 | – | – |
| **IVa** | 15.3 | 16.3 | – | – |
| **IVb** | 14.6 | 16.3 | – | – |
| **IVc** | 13.3 | 15.3 | – | – |
| **IVd** | 13.6 | 14.6 | **–** | **–** |
| **IVe** | **19.6** | **21.3** | **–** | **–** |
| **IVf** | 16.3 | 18.6 | – | – |
| **IVg** | 17.6 | 19.6 | – | – |
| **IVh** | 15.6 | 17.3 | **–** | **–** |
| **IVi** | 17.3 | 18.6 | **–** | **–** |
| Ciprofloxacin | 26.6 | 24.0 | 25.0 | 22.0 |

– No activity

[a] Values, including diameter of the well (8 mm), are means of three replicates

The significance of the bold values indicate the highest antimicrobial activity of that particular compound

2-thiobarbituric acids to give novel 3-benzyl-5-arylidene-2-thioxoimidazolidin-4-ones and 1,3-dibenzyl-5-arylidene-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-diones, respectively. High yields, mild reaction conditions, and environment friendliness of the protocol make it highly desirable. These derivatives were evaluated for their anti-microbial activities, of which, some have shown good antibacterial and antifungal activities.

## Experimental

All the chemicals used were of research grade and were used without further purification. Transmission electron microscopy for size and morphology characterization was obtained on TECNAI $G^2$ U-TWIN (300 kV). X-ray diffraction pattern was obtained on BRUKER D8. $^1$H NMR and $^{13}$C NMR spectra were recorded on JEOL JNM ECX-

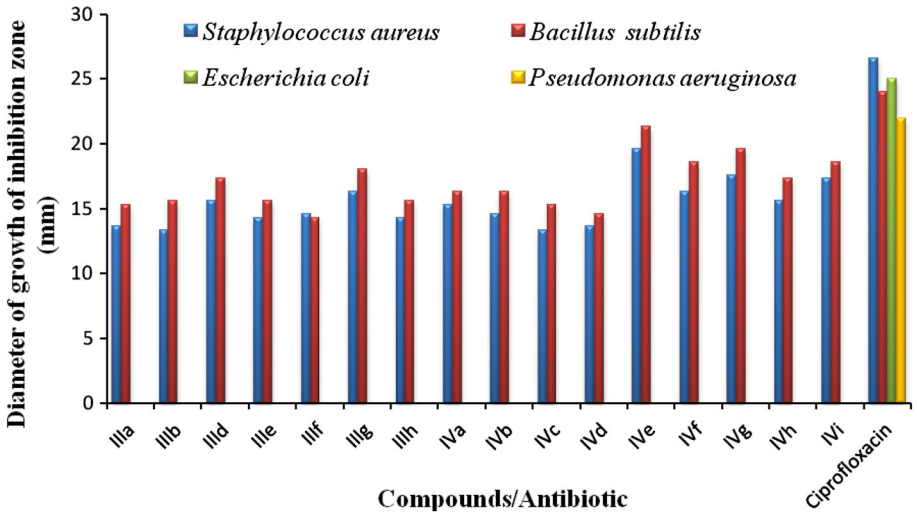



**Fig. 2** Comparison of diameter of growth of inhibition of compounds **III** and **IV** with standard drug

**Table 5** Minimum inhibitory concentration (MIC) (in μg/mL) of compounds using modified agar well diffusion method

| Compound | *Staphylococcus aureus* | *Bacillus subtilis* |
|---|---|---|
| **IIIa** | 256 | 128 |
| **IIIb** | 512 | 128 |
| **IIId** | 128 | 128 |
| **IIIe** | 256 | 128 |
| **IIIf** | 256 | 256 |
| **IIIg** | 128 | 64 |
| **IIIh** | 128 | 128 |
| **IVa** | 128 | 128 |
| **IVb** | 128 | 128 |
| **IVc** | 512 | 128 |
| **IVd** | 256 | 256 |
| **IVe** | **64** | **32** |
| **IVf** | 128 | 64 |
| **IVg** | **64** | 64 |
| **IVh** | 128 | 128 |
| **IVi** | 128 | 64 |
| Ciprofloxacin | 6.25 | 6.25 |

The significance of the bold values indicate the highest antimicrobial activity of that particular compound

400P (400 MHz) with DMSO-$d_6$ as solvent and TMS as internal standard. IR spectra were recorded on Perkin-Elmer FT-IR SPECTRUM-2000. Mass spectra were recorded on JEOL-AccuTOF JMS–T100 mass spectrometer having a DART source. Melting points were recorded on a Tropical Labequip apparatus and are uncorrected.

## Preparation of Ni nanoparticles

Ni nanoparticles were prepared as reported ( Khurana and Vij, 2011). To a $2 \times 10^{-4}$ M solution of $NiCl_2.6H_2O$ (0.0048 g) in ethylene glycol (100 mL) placed in a 250 mL R.B. flask fitted with a reflux condenser, polyethylene glycol i.e., PEG-4000 (0.024 g) was added. The mixture was stirred until complete dissolution of PEG. The solution was heated in an oil-bath maintained at 120°C. At this temperature, 0.36 g of $NaBH_4$ was added to the solution. The system was maintained under magnetic stirring at 120°C for 2 h. Formation of black-colored particles indicated the successful synthesis of Ni nanoparticles. The sample for TEM analysis was obtained by the addition of acetone to the Ni nanoparticles dispersion in ethylene

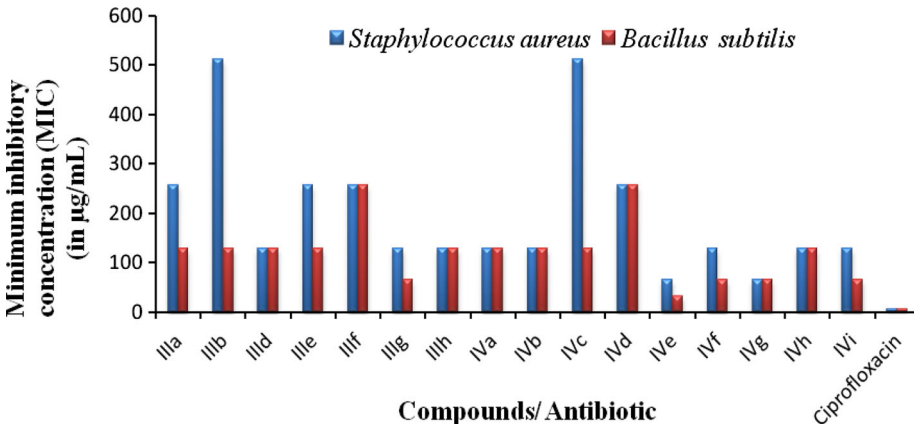



**Fig. 3** Comparison of MIC of compounds **III** and **IV** with standard drug

**Table 6** Antifungal activity of chemical compounds through poisoned food method

| Compound | Mycelial growth inhibition (%) | |
|---|---|---|
| | *Aspergillus niger* | *Aspergillus flavus* |
| **IIIa** | 35.5 | 37.7 |
| **IIIb** | 42.2 | 44.4 |
| **IIId** | 44.4 | 45.5 |
| **IIIe** | **48.8** | **51.1** |
| **IIIf** | 33.3 | 38.8 |
| **IIIg** | 35.5 | 38.8 |
| **IIIh** | 34.4 | 38.8 |
| **IVa** | 41.1 | 45.5 |
| **IVb** | 43.3 | 47.7 |
| **IVc** | 48.8 | 45.5 |
| **IVd** | 37.7 | 43.3 |
| **IVe** | **51.1** | **55.5** |
| **IVf** | 45.5 | 47.7 |
| **IVg** | 44.4 | 48.8 |
| **IVh** | 41.1 | 43.3 |
| **IVi** | 38.8 | 42.2 |
| Fluconazole | 81.1 | 77.7 |

The significance of the bold values indicate the highest antimicrobial activity of that particular compound

glycol, followed by centrifugation (6,000 rpm). The particles, so obtained, were washed free of any residual components using ethanol. Subsequently, a drop of methanol dispersion of Ni nanoparticles was placed on carbon-coated Cu grid (mesh size 300). Sample for the X-ray diffraction study was obtained by depositing a thin coating of the isolated Ni nanoparticles (dispersed in absolute ethanol) onto a glass plate followed by vacuum drying.

General procedure for the synthesis of 3-benzyl-5-arylidene-2-thioxoimidazolidin-4-ones (**IIIa–h**)

In a typical reaction, a mixture of aromatic aldehyde (1.0 equiv.), 2-thiohydantoin (1.0 equiv.), and benzyl bromide (1.0 equiv.) in Ni nanoparticles dispersion in ethylene glycol [2.0 mL of dispersion (0.0235 wt% of Ni)/0.1 g aldehyde] was stirred thoroughly in a 50 mL R. B. flask maintained at 70 °C. The reaction progress was monitored by TLC, using petroleum ether/ethyl acetate (60:40) as eluent. Upon completion, 15 mL of water was added to the reaction mixture, and the solid product was filtered at pump, washed with water, dried, and recrystallized from hot ethanol. The products were identified by spectral data.

*3-Benzyl-5-(4-nitrobenzylidene)-2-thioxoimidazolidin-4-one (**IIIa**)*

Yellow solid; Yield = 90 %; m.p. 233–235 °C; IR (KBr, $cm^{-1}$) $\upsilon_{max}$ = 3423, 3133, 2823, 1715, 1630, 1500, 1337, 1183, 1071; $^{1}$H NMR (400 MHz, DMSO-$d_6$) δ: 11.99 (s, 1H, NH), 8.40–8.36 (m, 2H, Ar–H), 8.23–8.20 (m, 2H, Ar–H), 7.49 (d, $J$ = 7.4 Hz, 2H, Ar–H), 7.33-7.21 (m, 3H, Ar–H), 6.78 (s, 1H, CH), 4.57 (s, 2H, $CH_2$); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ: 170.23(C=S), 167.31 (C=O), 146.82(CH, $C_4$Ar), 140.93(C, $C_1$Ar), 136.69($C_5$-CNH), 132.05(C, $C_1$Ph), 129.09(CH, $C_2$Ar, & $C_6$Ar), 128.58(CH, $C_3$Ph, $C_{Ph}$ & $C_5$Ph), 127.56(CH, $C_2$Ph & $C_6$Ph), 123.66(CH, $C_3$Ar & $C_5$Ar), 117.52(CH, C-CAr), 33.62($CH_2$, NCPh); MS (ESI) m/z calcd. = 339.07, found: 340.08 [$M^+$+H]. Anal. Calcd for $C_{17}H_{13}N_3O_3S$ : C, 60.17; H, 3.86; N, 12.38; S, 9.45 %. Found: C, 60.11; H, 3.83; N, 12.31; S, 9.40 %.

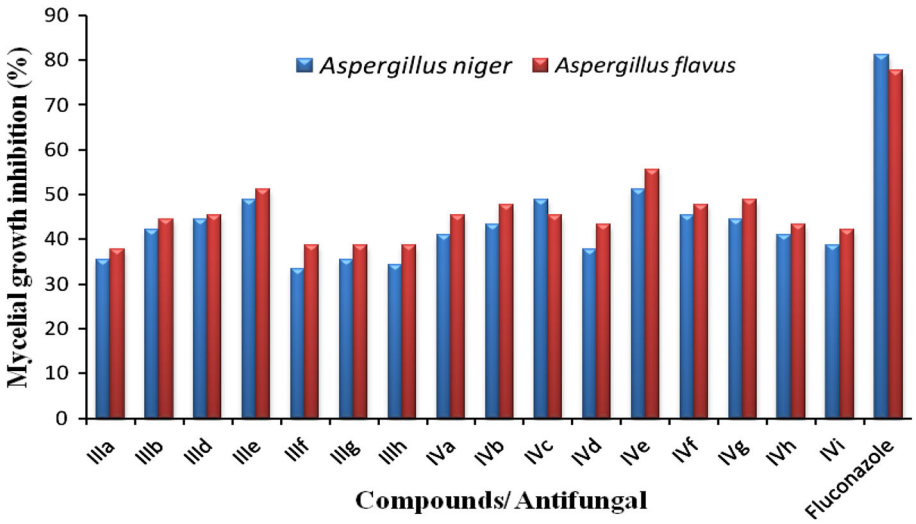



**Fig. 4** Comparison of antifungal activity of compounds **III** and **IV** with standard drug

*3-Benzyl-5-(2,4-dichlorobenzylidene)-2-thioxoimidazolidin-4-one (**IIIb**)*

Yellow solid; Yield = 91 %; m.p. 218–220 °C; IR (KBr, $cm^{-1}$) $\upsilon_{max}$ = 3392, 3087, 2809, 1712, 1630, 1504, 1412, 1184; $^1$H NMR (400 MHz, DMSO-$d_6$) δ: 12.04 (s, 1H, NH), 8.86 (d, $J$ = 8.8 Hz, 1H, Ar–H), 7.72–7.71 (m, 1H, Ar–H), 7.57–7.54 (m, 1H, Ar–H), 7.47 (d, $J$ = 7.6 Hz, 2H, Ar–H), 7.36–7.32 (m, 2H, Ar–H), 7.28–7.24 (m, 1H, Ar–H), 6.90 (s, 1H, CH), 4.55 (s, 2H, $CH_2$); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ: 170.19 (C=S), 166.87(C=O), 140.88 ($C_5$-CNH), 136.89 (CH, $C_1$Ph), 135.03(CCl-$C_2$Ar), 134.36(CCl-$C_4$Ar), 133.26(C, $C_1$Ar), 130.64(CH, $C_6$Ar), 129.22(CH, $C_3$Ar), 129.05(CH, $C_5$Ar), 128.56(CH, $C_3$Ph & $C_5$Ph), 127.75($C_2$Ph & $C_6$Ph), 127.52(C-$_4$Ph), 112.94(CH, C-CAr), 33.56($CH_2$, NCPh); MS (ESI) m/z calcd. = 362.00, found: 363.02 [$M^+$+H], 365.02 [($M^+$+H) + 2]; Anal. Calcd for $C_{17}H_{12}Cl_2N_2OS$ : C, 56.21; H, 3.33; Cl, 19.52; N, 7.71; S, 8.83 %. Found: C, 56.17; H, 3.30; Cl, 19.50; N, 7.67; S, 8.78 %.

*3-Benzyl-5-(3,4-dimethoxybenzylidene)-2-thioxoimidazolidin-4-one (**IIIc**)*

Yellow solid; Yield = 93 %; m.p. 195–197 °C; IR (KBr, $cm^{-1}$) $\upsilon_{max}$ = 3367, 3043, 2816, 1714, 1576, 1174; $^1$H NMR (400 MHz, DMSO-$d_6$) δ: 11.74 (s, 1H, NH), 8.17 (s, 1H, Ar–H), 7.60–7.57 (m, 1H, Ar–H), 7.49 (d, $J$ = 7.3 Hz, 2H, Ar–H), 7.34–7.25 (m, 3H, Ar–H), 7.01 (d, $J$ = 8.8 Hz, 1H, Ar–H), 6.73 (s, 1H, CH), 4.57 (s, 2H, $CH_2$), 3.79 (s, 3H, $OCH_3$), 3.69 (s, 3H, $OCH_3$); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ: 170.56(C, C=S), 162.25(C, C=O), 150.45(COMe, $C_3$Ar), 148.55(COMe, $C_4$Ar), 137.36(C, $C_5$ CNH), 137.18(C, $C_1$Ph), 128.87(C, $C_1$Ar), 128.62(CH, $C_3$Ph & $C_5$Ph), 127.54(CH, $C_2$Ph), 127.18(CH, $C_6$Ph), 126.13(CH, $C_4$Ph), 121.79 (CH, $C_6$Ar), 113.60(CH, $C_2$Ar & $C_5$Ar), 111.49(CH, C-CAr), 55.51(C, OMe), 55.09(C, OMe), 33.13($CH_2$, NCPh); MS (ESI) m/z calcd. = 354.10, found: 355.14 [$M^+$+H]; Anal. Calcd for $C_{19}H_{18}N_2O_3S$ : C, 64.39; H, 5.12; N, 7.90; S, 9.05 %. Found: C, 64.34; H, 5.08; N, 7.88; S, 9.02 %.

*3-Benzyl-5-(4-fluorobenzylidene)-2-thioxoimidazolidin-4-one (**IIId**)*

Pale yellow solid; Yield = 90 %; m.p. 195-197 °C; IR (KBr, $cm^{-1}$) $\upsilon_{max}$ = 3448, 3064, 2803, 1716, 1596, 1508, 1224, 1172; $^1$H NMR (400 MHz, DMSO-$d_6$) δ: 11.83 (s, 1H, NH), 8.28–8.24 (m, 2H, Ar–H), 7.50 (d, $J$ = 8.0 Hz, 2H, Ar–H), 7.35-7.23 (m, 5H, Ar–H), 6.76 (s, 1H, CH), 4.55 (s, 2H, $CH_2$); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ: 170.47(C, C=S), 164.14(C, C=O), 163.84(CF, $C_4$Ar), 161.36(CF, $C_4$Ar), 138.84(C, $C_5$, CNH), 137.09(C, $C_1$Ph), 133.78(CH, $C_2$Ar), 133.71(CH, $C_6$Ar), 130.94(C, $C_1$Ar), 129.04, 128.55, 127.49, 119.87(CH, C–Ar), 115.85(CH, $C_5$Ar), 115.63(CH, $C_3$Ar), 33.43($CH_2$, NCPh); MS (ESI) m/z calcd. = 312.07, found: 313.08 [$M^+$+H]; Anal. Calcd for $C_{17}H_{13}FN_2OS$ : C, 65.37; H, 4.19; F, 6.08; N, 8.97; S, 10.27 %. Found: C, 65.29; H, 4.13; F, 6.02; N, 8.92; S, 10.24 %.

*3-Benzyl-5-(3-nitrobenzylidene)-2-thioxoimidazolidin-4-one (**IIIe**)*

Yellow solid; Yield = 89 %; m.p. 228–230 °C; IR (KBr, $cm^{-1}$) $\upsilon_{max}$ = 3435, 3080, 2926, 1717, 1525, 1357, 1182; $^1$H NMR (400 MHz, DMSO-$d_6$) δ: 11.99 (s, 1H, NH), 9.30–9.28 (m, 1H, Ar–H), 8.45–8.43 (m, 1H, Ar–H), 8.19–8.17 (m, 1H, Ar–H), 7.72-7.68 (m, 1H, Ar–H), 7.55–7.53 (m, 2H, Ar–H), 7.33–7.25 (m, 3H, Ar–H), 6.87 (s, 1H, CH), 4.59 (s, 2H, $CH_2$); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ: 170.19(C, C=S), 166.32(C, C=O), 148.10(C-$NO_2$, $C_3$Ar), 140.87(C, $C_5$ CNH), 137.47(C, $C_1$Ph), 137.06(C, $C_1$Ar), 135.98(CH, $C_6$Ar), 130.12(CH, $C_4$Ar), 129.08(CH, $C_2$Ar), 128.58(CH, $C_3$Ph & $C_5$Ph), 127.56(CH, $C_2$Ph & $C_6$Ph), 125.04(CH, $C_4$Ph), 123.55(CH, $C_5$Ar), 117.90(CH, C–Ar), 33.41($CH_2$, NCPh); MS (ESI) m/z calcd. = 339.07, found: 340.08 [$M^+$+H]; Anal. Calcd for $C_{17}H_{13}N_3O_3S$ : C, 60.17; H, 3.86; N, 12.38; S, 9.45 %. Found: C, 60.13; H, 3.82; N, 12.33; S, 9.42 %.

*4-((1-Benzyl-5-oxo-2-thioxoimidazolidin-4-ylidene)methyl)benzonitrile (**IIIf**)*

Yellow solid; Yield = 94 %; m.p. 230–232 °C; IR (KBr, $cm^{-1}$) $\upsilon_{max}$ = 3447, 3128, 2222, 1717, 1632, 1501, 1406, 1182; $^1$H NMR (400 MHz, DMSO-$d_6$) δ: 11.99 (s, 1H, NH), 8.33 (d, $J$ = 8.0 Hz, 2H, Ar–H), 7.86 (d, $J$ = 8.0 Hz, 2H, Ar–H), 7.50 (d, $J$ = 7.2 Hz, 2H, Ar–H), 7.36–7.32 (m, 2H, Ar–H), 7.27–7.26 (m, 1H, Ar–H), 6.77 (s, 1H, CH), 4.56 (s, 2H, $CH_2$); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ: 170.24(C, C=S), 166.64(C, C=O), 141.31(C, $C_1$Ar), 138.87(C, $C_1$Ar), 136.93(C, $C_1$Ph), 132.44, 132.33, 131.66, 129.83, 129.08, 128.60, 127.55, 118.89, 118.23, 110.93, 105.81, 33.59($CH_2$, NCPh); MS (ESI) m/z calcd. = 319.08, found: 320.08 [$M^+$+H]; Anal. Calcd for $C_{18}H_{13}N_3OS$ : C, 67.69; H, 4.10; N, 13.16; S, 10.04 %. Found: C, 67.64; H, 4.04; N, 13.11; S, 10.00 %.

*3-((1-Benzyl-5-oxo-2-thioxoimidazolidin-4-ylidene)methyl)benzonitrile (**IIIg**)*

Yellow solid; Yield = 93 %; m.p. 203–205 °C; IR (KBr, $cm^{-1}$) $\upsilon_{max}$ = 3433, 3126, 2925, 2233, 1717, 1639, 1500, 1187; $^1$H NMR (400 MHz, DMSO-$d_6$) δ: 11.96 (s, 1H, NH), 8.65 (s, 1H, Ar–H), 8.44–8.42 (m, 1H, Ar–H), 7.81–7.89 (m, 1H, Ar–H), 7.65–7.61 (m, 1H, Ar–H), 7.53–7.51 (m, 2H, Ar–H), 7.36–7.33 (m, 2H, Ar–H), 7.27–7.24 (m, 1H, Ar–H), 6.76 (s, 1H, CH), 4.55 (s, 2H, $CH_2$); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ: 170.16(C, C=S), 166.07(C, C=O), 140.64(C,

$C_1$NH), 137.10(C, $C_1$Ph), 135.58(C, $C_1$Ar), 135.50(CH, $C_4$Ar), 134.22(CH, $C_6$Ar), 132.30(CH, $C_2$Ar), 129.85(CH, $C_5$Ar), 128.98(CH, $C_3$Ar & CH, $C_5$Ar), 128.52(CH, $C_2$Ph & CH, $C_6$Ph), 127.47(CH, $C_4$Ph), 118.58(C, C≡N), 118.00(CH, C–Ar), 111.81(C, $C_3$Ar), 33.53($CH_2$, NCPh,); MS (ESI) m/z calcd. = 319.08, found: 320.08 [$M^+$+H]; Anal. Calcd for $C_{18}H_{13}N_3OS$ : C, 67.69; H, 4.10; N, 13.16; S, 10.04 %. Found: C, 67.62; H, 4.07; N, 13.10; S, 9.98 %.

*3-Benzyl-5-(4-(dimethylamino)benzylidene)-2-thioxoimidazolidin-4-one (**IIIh**)*[26]

Orange solid; Yield = 92 %; m.p. 195–200 °C; IR (KBr, $cm^{-1}$) $\upsilon_{max}$ = 3435, 3005, 2820, 1692, 1586, 1525, 1433, 1367, 1163; $^1$H NMR (400 MHz, DMSO-$d_6$) δ: 11.55 (s, 1H, NH), 8.05 (d, $J$ = 8.8 Hz, 2H, Ar–H), 7.50 (d, $J$ = 7.3 Hz, 2H, Ar–H), 7.35–7.31 (m, 2H, Ar–H), 7.27–7.23 (m, 1H, Ar–H), 6.75 (d, $J$ = 8.8 Hz, 2H, Ar–H), 6.67 (s, 1H, CH), 4.53 (s, 2H, $CH_2$), 2.99 (s, 6H, N-$CH_3$); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ: 170.52(C, C=S), 159.27(C, C=O), 151.10(C, $C_4$Ar), 137.42(C, $C_5$NH), 135.24(C, $C_1$Ph), 133.39, 129.03(CH, $C_2$Ar & CH, $C_6$Ar), 128.55(CH, $C_3$Ph & CH, $C_5$Ph), 127.44(CH, $C_4$Ph), 123.21(C, $C_1$Ar), 121.70(CH, $C_3$Ar & $C_5$Ar), 111.71(C, C–Ar), 33.23($CH_2$, NCPh); Anal. Calcd for $C_{19}H_{19}N_3OS$ : C, 67.63; H, 5.68; N, 12.45; S, 9.50 %. Found: C, 67.58; H, 5.63; N, 12.44; S, 9.47 %.

General procedure for the synthesis of 1,3-dibenzyl-5-arylidene-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-diones (**IVa–i**)

In a typical reaction, a mixture of aromatic aldehyde (1.0 equiv.), 2-thiobarbituric acid (1.0 equiv.), benzyl bromide (2.0 equiv.), and Ni nanoparticles dispersion in ethylene glycol [2.0 mL of dispersion (0.0235 wt% of Ni)/0.1 g aldehyde] was stirred thoroughly in a 50 mL R. B. flask maintained at 70 °C. The reaction progress was monitored by thin column chromatography (TLC), using petroleum ether/ethyl acetate (60:40) as eluent. Upon completion, 15 mL of water was added to the reaction mixture, and the solid product was filtered at pump, washed with water, dried, and recrystallized from hot ethanol. The products were identified by spectral data.

*1,3-Dibenzyl-5-(4-nitrobenzylidene)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione (**IVa**)*

Colorless solid; Yield = 92 %; m.p. 245–247 °C; IR (KBr, $cm^{-1}$) $\upsilon_{max}$ = 2909, 1627, 1548, 1515, 1419, 1345, 1302, 1232, 1096; $^1$H NMR (400 MHz, DMSO-$d_6$) δ: 8.09 (d, $J$ = 8.0 Hz, 2H, Ar–H), 7.45–7.43 (m, 4H, Ar–H), 7.33–7.29 (m, 5H, Ar–H), 7.28–7.23 (m, 3H, Ar–H), 6.10 (s, 1H, CH), 4.40 (s, 4H, $CH_2$); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ: 166.34(C, C=S), 159.46(C, C=O), 149.47(C-$NO_2$, $C_4$Ar), 145.67(CH, C-CAr), 137.07(C, $C_1$Ar), 130.67(CH, C=CAr), 129.19(CH, $C_3$Ph & CH, $C_5$Ph), 128.58(CH, $C_2$Ph & CH, $C_6$Ph), 127.99(CH, CAr), 127.45(CH, $C_4$Ph), 123.56(CH, CAr), 123.30(CH, CAr), 98.91(C, C = CAr), 33.47($CH_2$, NCPh), 32.87($CH_2$, NCPh); MS (ESI) m/z calcd. = 457.11, found: 458.14 [$M^+$+H]; Anal. Calcd for $C_{25}H_{19}N_3O_4S$ : C, 65.63; H, 4.19; N, 9.18; S, 7.01 %. Found: C, 65.60; H, 4.14; N, 9.13; S, 6.99 %.

*1,3-Dibenzyl-5-(4-fluorobenzylidene)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione (**IVb**)*

Colorless solid; Yield = 88 %; m.p. 255–257 °C (d); IR (KBr, $cm^{-1}$) $\upsilon_{max}$ = 3027, 2926, 2853, 1623, 1552, 1507, 1407, 1296, 1227, 1065; $^1$H NMR (400 MHz, DMSO-$d_6$) δ: 7.32 (d, $J$ = 7.3 Hz, 4H, Ar–H), 7.31 (t, $J$ = 7.3 Hz, 4H, Ar–H), 7.27–7.23 (m, 2H, Ar–H), 7.05–7.00 (m, 4H, Ar–H), 6.02 (s, 1H, CH), 4.39 (s, 4H, $CH_2$); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ: 166.36(C, C=S), 161.64(C–F, CAr) 159.24(C, C=O), 137.07(C, $C_1$Ph), 136.18(CH, C=CAr), 129.15(CH, $C_3$Ph & CH, $C_5$Ph), 128.54(CH, $C_2$Ph & CH, $C_6$Ph), 128.34(CH, CAr), 128.26(CH, CAr), 127.40(CH, $C_4$Ph), 114.76(CH, CAr), 114.55(CH, CAr), 99.62(C, C=CAr), 33.41($CH_2$, NCPh), 31.50($CH_2$, NCPh); MS (ESI) m/z calcd. = 430.12, found: 431.14 [$M^+$+H]; Anal. Calcd for $C_{25}H_{19}FN_2O_2S$ : C, 69.75; H, 4.45; F, 4.41; N, 6.51; S, 7.45 %. Found: C, 69.70; H, 4.39; F, 4.38; N, 6.44; S, 7.42 %.

*1,3-Dibenzyl-5-(3-nitrobenzylidene)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione (**IVc**)*

Colorless solid; Yield = 90 %; m.p. 225–227 °C; IR (KBr, $cm^{-1}$) $\upsilon_{max}$ = 2924, 1624, 1560, 1420, 1350, 1300, 1094; $^1$H NMR (400 MHz, DMSO-$d_6$) δ: 8.03 (d, $J$ = 7.3 Hz, 1H, Ar–H), 7.77 (s, 1H, Ar–H), 7.55–7.49 (m, 2H, Ar–H), 7.44 (d, $J$ = 6.6 Hz, 4H, Ar–H), 7.33–7.23 (m, 6H, Ar–H), 6.07 (s, 1H, CH), 4.40 (s, 4H, $CH_2$); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ: 166.24(C, C=S), 166.03, 147.81(C-$NO_2$, $C_4$Ar), 143.39(CH, C-CAr), 137.06(CH, C=CAr), 133.93($C_1$, CAr), 129.61(CH, $C_3$Ph & CH, $C_5$Ph), 129.18(CH, $C_2$Ph & CH, $C_6$Ph), 128.57(CH, CAr), 127.44(CH, CAr), 121.08(CH, CAr), 120.88(CH, CAr), 98.76(C, C=CAr), 33.46($CH_2$, NCPh), 30.71; MS (ESI) m/z calcd. = 457.11, found: 458.14 [$M^+$+H]; Anal. Calcd for $C_{25}H_{19}N_3O_4S$ : C, 65.63; H, 4.19; N, 9.18; S, 7.01 %. Found: C, 65.57; H, 4.12; N, 9.15; S, 6.97 %.

*1,3-Dibenzyl-5-(2,5-dimethoxybenzylidene)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione (**IVd**)*

Yellow solid; Yield = 89 %; m.p. 150–152 °C; IR (KBr, $cm^{-1}$) $\upsilon_{max}$ = 2929, 1624, 1560, 1495, 1424, 1293, 1225; $^1$H NMR (400 MHz, DMSO-$d_6$) δ: 7.42 (d, $J$ = 7.3 Hz, 4H, Ar–H), 7.32–7.28 (m, 4H, Ar–H), 7.26–7.22 (m, 2H, Ar–H), 6.76 (d, $J$ = 8.7 Hz, 1H, Ar–H), 6.69–6.66 (m, 1H, Ar–H), 6.54–6.53 (m, 1H, Ar–H), 5.95 (s, 1H, CH), 4.37 (s, 4H, $CH_2$), 3.61 (s, 3H, OMe), 3.49 (s, 3H, OMe); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ: 152.66(C, COMe), 151.47 (C, COMe), 137.32(C, $C_1$Ph), 130.78, 129.12(CH, $C_3$Ph & CH, $C_5$Ph), 128.52(CH, $C_2$Ph & CH, $C_6$Ph), 127.38 (CH, $C_4$Ph), 116.03 (CH, CAr), 111.79 (CH, CAr), 109.74(CH, CAr), 99.69(C, C=CAr), 56.10($CH_3$, OMe), 55.10($CH_3$, OMe), 33.36 ($CH_2$, NCPh), 30.69($CH_2$, NCPh); MS (ESI) m/z calcd. = 472.15, found: 473.13 [$M^+$+H]; Anal. Calcd for $C_{27}H_{24}N_2O_4S$ : C, 68.62; H, 5.12; N, 5.93; S, 6.79 %. Found: C, 68.59; H, 5.10; N, 5.88; S, 6.75 %.

*1,3-Dibenzyl-5-(4-isopropylbenzylidene)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione (**IVe**)*

Colorless solid; Yield = 89 %; m.p. 250 °C (d); IR (KBr, $cm^{-1}$) $\upsilon_{max}$ = 2926, 1624, 1560, 1411, 1295, 1266, 1068; $^1$H NMR (400 MHz, DMSO-$d_6$) δ: 7.43 (d, $J$ = 7.3 Hz, 4H, Ar–H), 7.33–7.29 (m, 4H, Ar–H), 7.27–7.23 (m, 2H, Ar–H), 7.07 (d, $J$ = 8.0 Hz, 2H, Ar–H), 6.89 (d, $J$ = 7.3 Hz, 2H, Ar–H), 6.02 (s, 1H, CH), 4.39 (s, 4H, $CH_2$), 2.84-2.76 (m, 1H, CH), 1.15 (d, $J$ = 7.3 Hz, 6H, $CH_3$); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ: 166.53(C, C=S), 158.78(C, C=O), 145.41(CH, C-CAr), 137.42($C_1$, CAr), 137.09($C_1$, CPh), 129.15(CH, $C_3$Ph & CH, $C_5$Ph), 128.55(CH, $C_2$Ph & CH,$C_6$Ph), 127.41(CH, $C_4$Ph), 126.38(CH, CAr), 125.97(CH, CAr), 99.81(C, C=CAr), 33.43($CH_2$, NCPh), 32.93($CH_2$, NCPh), 31.59(CH, CH(Me)$_2$), 23.96($CH_3$, CH(Me)$_2$); MS (ESI) m/z calcd. = 454.17, found: 455.19 [$M^+$+H]; Anal. Calcd for $C_{28}H_{26}N_2O_2S$ : C, 73.98; H, 5.76; N, 6.16; S, 7.05 %. Found: C, 73.92; H, 5.73; N, 6.12; S, 6.99 %.

*1,3-Dibenzyl-5-(2-methylbenzylidene)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione (**IVf**)*

Colorless solid; Yield = 91 %; m.p. 248–250 °C (d); IR (KBr, $cm^{-1}$) $\upsilon_{max}$ = 2927, 1610, 1549, 1420, 1284, 1237, 1066; $^1$H NMR (400 MHz, DMSO-$d_6$) δ: 7.42 (d, $J$ = 7.3 Hz, 4H, Ar–H), 7.32–7.23 (m, 6H, Ar–H), 7.03 (s, 4H, Ar–H), 5.86 (s, 1H, CH), 4.37 (s, 4H, $CH_2$), 2.00 (s, 3H, $CH_3$); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ: 166.43(C, C=S), 166.02(C, C=O), 139.47(CH, C-CAr), 137.14($C_1$, CPh), 135.50($C_1$, CAr), 130.16(C, CAr), 129.12(CH, $C_3$Ph & CH, $C_5$Ph), 128.51(CH, $C_2$Ph & CH, $C_6$Ph), 127.78(CH, CAr), 127.38(CH, $C_4$Ph), 125.65(CH, CAr), 125.17(CH, CAr), 99.52(C, C=CAr), 33.40($CH_2$, NCPh), 31.55, 19.01($CH_3$, C(Me)); MS (ESI) m/z calcd. = 426.14, found: 427.16 [$M^+$+H]; Anal. Calcd for $C_{26}H_{22}N_2O_2S$ : C, 73.21; H, 5.20; N, 6.57; S, 7.52 %. Found: C, 73.16; H, 5.13; N, 6.49; S, 7.47 %.

*1,3-Dibenzyl-5-(2-nitrobenzylidene)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione (**IVg**)*

Colorless solid; Yield = 90 %; m.p. 220–224 °C; IR (KBr, $cm^{-1}$) $\upsilon_{max}$ = 3061, 1653, 1545, 1395, 1363, 1286, 1240, 1063; $^1$H NMR (400 MHz, DMSO-$d_6$) δ: 7.67 (d, $J$ = 6.6 Hz, 1H, Ar–H), 7.53–7.49 (m, 1H, Ar–H), 7.42 (d, $J$ = 7.3 Hz, 4H, Ar–H), 7.39–7.35 (m, 1H, Ar–H), 7.31 (t, $J$ = 7.3 Hz, 4H, Ar–H), 7.26-7.22 (m, 3H, Ar–H), 6.21 (s, 1H, CH), 4.36 (s, 4H, $CH_2$); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ: 206.48(C, C=S), 165.47(C, C=O), 149.50(CH, C-CAr), 137.20(C-$NO_2$, $C_2$Ar), 135.08($C_1$, CPh), 131.88, 129.81(CH, $C_3$Ph & CH, $C_5$Ph), 129.14(CH, $C_2$Ph & CH, $C_6$Ph), 128.53(CH, CAr), 127.38(CH, $C_4$Ph), 126.97(CH, CAr), 123.77(CH, CAr), 98.03(C, C=CAr), 33.35($CH_2$, NCPh), 30.70($CH_2$, NCPh); MS (ESI) m/z calcd. = 457.11, found: 458.12 [$M^+$+H]; Anal. Calcd for $C_{25}H_{19}N_3O_4S$ : C, 65.63; H, 4.19; N, 9.18; S, 7.01 %. Found: C, 65.59; H, 4.16; N, 9.11; S, 6.97 %.

*1,3-Dibenzyl-5-(naphthalen-2-ylmethylene)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione (**IVh**)*

Colorless solid; Yield = 87 %; m.p. 205–207 °C; IR (KBr, $cm^{-1}$) $\upsilon_{max}$ = 2924, 2854, 1618, 1546, 1423, 1295, 1274, 1220; $^1$H NMR (400 MHz, DMSO-$d_6$) δ: 7.83–7.75 (m, 2H, Ar–H), 7.46–7.39 (m, 8H, Ar–H), 7.32 (t, $J$ = 7.3 Hz, 4H, Ar–H), 7.28–7.24 (m, 2H, Ar–H), 7.18 (d, $J$ = 8.8 Hz, 1H, Ar–H), 6.21 (s, 1H, CH), 4.42 (s, 4H, $CH_2$); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ: 166.60(C, C=S), 158.93(C, C=O) 137.96(CH, C-CAr), 137.09(C, $C_1$Ph), 133.02(C, CNp), 131.57(C, CNp), 129.17(CH, $C_3$Ph & $C_5$Ph), 128.56(CH, $C_2$Ph & $C_6$Ph), 127.55(C, CNp), 127.42(CH, $C_4$Ph), 127.25(C, CNp), 125.85(C, CNp), 125.20(C, CNp), 124.17(C, CNp), 99.69(C, C=CAr), 33.46($CH_2$, NCPh), 32.40($CH_2$, NCPh); MS (ESI) m/z calcd. = 462.14, found: 463.13 [$M^+$+H]; Anal. Calcd for $C_{29}H_{22}N_2O_2S$ : C, 75.30; H, 4.79; N, 6.06; S, 6.93 %. Found: C, 75.27; H, 4.71; N, 6.02; S, 6.90 %.

*1,3-Dibenzyl-5-(3-chlorobenzylidene)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione (**IVi**)*

Colorless solid; Yield = 92 %; m.p. 240 °C (d); IR (KBr, $cm^{-1}$) $\upsilon_{max}$ = 2924, 2854, 1618, 1546, 1423, 1295, 1274, 1220; $^1$H NMR (400 MHz, DMSO-$d_6$) δ: 7.44 (d, $J$ = 7.3 Hz, 4H, Ar–H), 7.31 (t, $J$ = 7.3 Hz, 4H, Ar–H),

7.27–7.19 (m, 4H, Ar–H), 6.97 (s, 2H, Ar–H), 6.03 (s, 1H, CH), 4.40 (s, 4H, $CH_2$); $^{13}C$ NMR (100 MHz, DMSO-$d_6$) δ: 166.48(C, C=S), 159.04(C, C=O), 143.23(CH, C-CAr), 137.04(C, $C_1$Ph), 132.84(C–Cl, $C_3$Ar), 129.94(C, $C_1$Ar), 129.17(CH, $C_3$Ph & CH, $C_5$Ph), 128.55(CH, $C_2$Ph & CH, $C_6$Ph), 127.43(CH, $C_4$Ph), 126.21(CH, CAr), 125.64(CH, CAr), 125.48(CH, CAr), 99.11(C, C=CAr), 33.46 ($CH_2$, NCPh), 32.07($CH_2$, NCPh); MS (ESI) m/z calcd. = 446.09, found: 447.09 [$M^+$+H]; Anal. Calcd for $C_{25}H_{19}ClN_2O_2S$ : C, 67.18; H, 4.28; Cl, 7.93; N, 6.27; S, 7.17 %. Found: C, 67.09; H, 4.23; Cl, 7.89; N, 6.21; S, 7.14 %.

## Anti-microbial assay

### *Antibacterial activity*

The antibacterial activity of 16 compounds was evaluated by the agar well diffusion method. All the microbial cultures were adjusted to 0.5 McFarland standard, which is visually comparable to a microbial suspension of approximately $1.5 \times 10^6$ cfu/mL. 20 mL of Mueller–Hinton agar medium was poured into each Petri plate, and plates were swabbed with 100 µL inocula of the test microorganisms and kept for 15 min for adsorption. Using sterile cork borer of 8-mm diameter, wells were bored into the seeded agar plates and these were loaded with a 100 µL volume with concentration of 2.0 mg/mL of each compound reconstituted in the dimethylsulfoxide (DMSO). All the plates were incubated at 37 °C for 24 h. Antibacterial activity of each compound was evaluated by measuring the zone of growth inhibition against the test organisms with zone reader (HiAntibiotic zone scale). DMSO was used as a negative control whereas Ciprofloxacin was used as positive control. This procedure was performed in three replicate plates for each organism, and the mean values of the diameter of inhibition zones ± standard deviations were calculated (Aneja *et al*., 2011).

### *Determination of minimum inhibitory concentration (MIC) of chemical compounds*

MIC is the lowest concentration of an anti-microbial compound that will inhibit the visible growth of a microorganism after overnight incubation. MIC of the various compounds against bacterial strains was tested through a modified agar well diffusion method (Aneja *et al*., 2011). In this method, a two-fold serial dilution of each chemically synthesized compound was prepared by first reconstituting the compound in DMSO followed by dilution in sterile distilled water to achieve a decreasing concentration range of 512–1 µg/mL. A 100 µL volume of each dilution was introduced into wells (in triplicate) in the agar plates already seeded with 100 µL of standardized inoculum ($10^6$ cfu/mL) of the test microbial strain. All test plates were incubated aerobically at 37 °C for 24 h and observed for the inhibition zones. MIC, taken as the lowest concentration of the chemical compound that completely inhibited the growth of the microbe, showed by a clear zone of inhibition, was recorded for each test organism. Ciprofloxacin was used as positive control.

### *Antifungal activity*

The antifungal activity of 16 compounds was evaluated by poisoned food technique (Al- Burtamani *et al*., 2005). The molds were grown on Sabouraud dextrose agar (SDA) at 25 °C for 7 days and used as inocula. The 15 mL of molten SDA (45 °C) was poisoned by the addition of 100 µL volume of each compound having concentration of 2.0 mg/mL reconstituted in the DMSO, poured into a sterile Petri plate and allowed it to solidify at room temperature. The solidified poisoned agar plates were inoculated at the center with fungal plugs (8 mm diameter) obtained from the colony margins and incubated at 25 °C for 7 days. DMSO was used as the negative control whereas Fluconazole was used as the positive control. The experiments were performed in triplicates. Diameter of fungal colonies was measured and expressed as percent mycelial inhibition.

$$\text{Percent inhibition of myelial growth} = (\text{dc} - \text{dt})/\text{dc} \times 100,$$

dc = average diameter of fungal colony in negative control, setsdt = average diameter fungal colony in experimental sets.

**Acknowledgments** Sneha is thankful to CSIR, New Delhi, India for the award of Junior and Senior Research Fellowship.

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