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Synthesis of some novel 6,6'-(1,4-phenylene)bis(4-phenylpyrimidin-2-ol/-2-amine/2-thiol catalyzed by $MgFe_2O_4$ and evaluation of their antimicrobial activities

S. Bansal[a] and P. P. Singh[a]*

[a]*Department of Chemistry, GLA University, NH-2 Delhi Mathura highway, Chaumuhan, Mathura 281406 India*

** Corresponding author:*
prabal.singh@gla.ac.in
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Abstract

Novel bisheterocycles **4-6** have been synthesized by green approach using cheaper, reusable, non toxic magnetically separable $MgFe_2O_4$ as nano catalyst. Bis chalcone and binucleophiles were treated on $MgFe_2O_4$ heterogenous catalyst in refluxing ethanol to afford the novel bis heterocycles **4-6** in very good yields with very less reaction time. The newly synthesized compounds were characterized by NMR (^1H & ^{13}C), FTIR and mass spectroscopy, thereafter evaluated for their antibacterial activities against *Bacillus subtilis* (MTCC No.121), *Escherichia coli* (MTCC No.118), *Staphylococcus aureus* (MTCC No. 96) and *Salmonella typhi* (MTCC No. 98) while antifungal activities were tested against *Aspergillus niger* (MTCC No. 281) and *Candida albicans* (MTCC No. 2479) using disc diffusion method. The reported novel compounds exhibited a good range of antimicrobial activities, opening plethora of oppournities in the field of research in antibiotics.

Keywords: *Intermolecular Interaction – hydrogen bonding; Thermal agitation factors; DFT.*

1.Introduction

Structural sub units of five and six membered heterocycles that are abundant in nature exist in many natural products like vitamins, hormones, amino acids, alkaloids, haemoglobin and antibiotics [1]. Among the various heterocyclic systems, pyrimidines are most important six membered heterocycles containing two nitrogen atoms at position 1 and 3. Pyrimidine derivatives have shown anticancer [2], antimicrobial [3], anticonvulsant [4], antiviral [5], antineoplasty [6], antihistalmic [7], antidiabetic [8], antiallergic [9], antipyretic [10] and antihypertensive [11] activities. On the other hand classes of pyrimidines possess a broad spectrum of biological effectiveness such as antitubercular [12], calcium channel blockers [13] and many classes of chemotherapeutic. Despite of remarkable development in the field of pharmaceutical sciences, various infectious diseases caused by bacteria, fungi, viruses and parasites are still a big challenge to public health. Emergence of widespread drug resistance in the above microorganisms has led a great impact on the health of the people of developing countries in particular. The development of drug resistance and appearance of undesirable side effect of certain antibiotics has forced the researcher to look for new antimicrobial agents, with a goal to discover new chemical structural frameworks which may overcome the above mentioned shortcomings. Literature reveals that many efforts has been made towards the development of simpler and high yielding methods for synthesis of pyrimidines derivatives in presence of various reagents like NaOH [14], $ZnCl_2$ [15], $Pd(PPh_3)_2Cl_2$ [16], Tf_2O/2-chloropyridine [17], HPA [18], KO^tBu [19], $POCl_3$-K_2CO_3 [20] using toxic solvent at one or other stage of the synthesis. Such methodology may not be industrially and environmentally acceptable. Under this vision, realizing the importance six membered heterocyclic compounds as antimicrobial agents and our keen interest in the development of green method in synthesis [21-22], we herein report three novel bis-pyrimidines analogues like 6,6'-(1,4-phenylene)bis(4-phenylpyrimidin-2-ol), 6,6'-(1,4-phenylene)bis(4-phenylpyrimidin-2-amine) and (6,6'-(1,4-phenylene)bis(4-phenylpyrimidin-2-thiol) efficiently synthesized by simple approach by using cheaper, reusable, non toxic magnetically separable $MgFe_2O_4$ as nano catalyst with reduced reaction time. The potency of these newly synthesized compounds were examined against both for gram (+) and gram (-) stain bacteria along with their antifungal bioassay after complete characteristic of synthesized compounds.

2. Materials and methods

2.1. Experimental section

All the solvents and reagents were used as such as supplied from Merck (Darmstadt, Germany) and S.D. Fine-chem (Mumbai, India). FTIR spectra were recorded in KBr on a Schimadzu FTIR 8401 spectrometer. ^1H and ^{13}C spectra were recorded on a Bruker DRX 300 spectrometer operating at 300MHz for ^1H NMR and 75MHz for ^{13}C NMR solutions in $CDCl_3$ and DMSOd_6. The ESI mass spectra were measured on waters UPLC-TDQ spectrometer. TLC was performed on silica coated glass plates; spots were developed in I_2 Chamber or visualized in UV chamber. The morphology of the catalyst was studied by high resolution electron microscopy HRTEM-300 KV Technai G2 30 S TWIN with gold coating equipped with energy dispersive X-ray spectroscopy. Melting points were recorded on digital Scientech instrument using capillary method and are uncorrected.

2.2. General procedure for the synthesized of (4-6)

Bispyrimidine analogues **(4-6)** were synthesized by refluxing 3,3'-(1,4- phenylene)bis(1-phenylprop-2-en-1-one) **3** with bifunctional nucleophiles like urea, guanidine nitrate and thiourea respectively in presence of 10 mmol% of $MgFe_2O_4$ MNP's (See ESI) as catalyst for the respective period of time. The progress of the reaction was monitored by TLC. On completion, the reaction mixture was allowed to cool at room temperature, the catalyst was separated by using external magnet and the supernatant liquid was evaporated up to the dryness under reduced pressure to obtain a crude solid product. The solid obtained was stirred in water, filtered and recrystallized from EtOAc:Hexane solutions

or passed through column chromatography if necessary. The structure of the synthesized heterocycles was characterized by NMR (^{1}H & ^{13}C) spectra, mass and IR spectroscopy (see ESI). Looking for the advantages of developed strategy, we compared our protocol with various published reports [23-27] and it has been found that the catalyst reduced the reaction time drastically from 2-12 hours (in the reported process) to 30-120 minutes for all the reaction to obtain the product in good yields.

(6,6'-(1,4phenylene)bis(4-phenylpyrimidine-2-ol)) **(4)**

Red orange solid, mp >300°C, Yield= 86%; R_f =0.43 (2:8 EtOAc/hexane); IR (cm^{-1}): 3422, 3004, 2259, 2190, 1178, 1013, 923; ^{1}H NMR (300 MHz, $CDCl_3$): 7.79-7.67 (m, 1H), 7.38-7.17 (m, 6H); ^{13}C NMR (75MHz, $CDCl_3$): 174.84, 129.29, 128.98, 128.21, 126.99, 126.80; Mass ESI m/z (%) M^{+}+1=419. Anal. calcd. for $C_{26}H_{18}N_4O_2$: C, 74.63; H, 4.34; N, 13.39. Found: C, 74.78; H, 4.24; N, 13.45.

[6,6'-(1,4-phenylene)bis(4-phenylpyridine-2-amine)] **(5)**

Bright yellow crystal, mp 220-221°C, Yield=85%, R_f=0.42(2:8 EtOAc/hexane); IR (cm^{-1}): 3309, 3043, 1912, 1572. ^{1}H NMR (300 MHz, $CDCl_3$): 8.09 (s, 1H) 7.46-7.39 (m, 5H); 7.12-6.75 (m, 2H). Mass ESI m/z (%) M^{+}+1=417, M^{+}+2=418. Anal. calcd. for $C_{26}H_{20}N_6$: C, 74.98; H, 4.84; N, 20.18. Found: C, 74.76; H, 4.72; N, 20.24.

(6,6'-(1,4phenylene)bis(4-phenylpyrimidine-2-thiol)) **(6)**

Pink solid, mp 270-72°C; Yield= 90%; R_f =0.47 (2:8 EtOAc/hexane); IR (cm^{-1}): 3026, 2827, 1972, 1619, 1437, 1339, 1214, 1102; ^{1}H NMR (300 MHz, $CDCl_3$): 8.03 (d, J = 5.4, 1H); 7.83-7.50 (m, 6H); ^{13}C NMR (75MHz, $CDCl_3$): 190.51, 143.78, 138.29, 137.12, 133.18, 129.19, 128.92, 128.75, 123.32. Mass ESI m/z (%) M^{+}-phenyl) = 339. Anal. calcd. for $C_{26}H_{18}N_4S_2$: C, 69.31; H, 4.03; N, 12.43; S, 14.23. Found: C, 69.43; H, 4.10; N, 12.33.

3. Results and Discussions

3.1 *Characterization of catalyst*

The high resolution electron microscopy (HRTEM) of $MgFe_2O_4$ MNP'S is shown in Figure 1. The particle size of the magnesium ferrite NP's sample is typically in the range of 100-200nm. The small crystallize are irregular in shape and are attached to each other along the grain boundaries. The material was verified by XRD data which matched very well with standard data. Size of crystallite was found by 100nm from analysis of XRD profile by Debye Sheerer equation surface area was found to be $12m^2$/gm Figure 2.

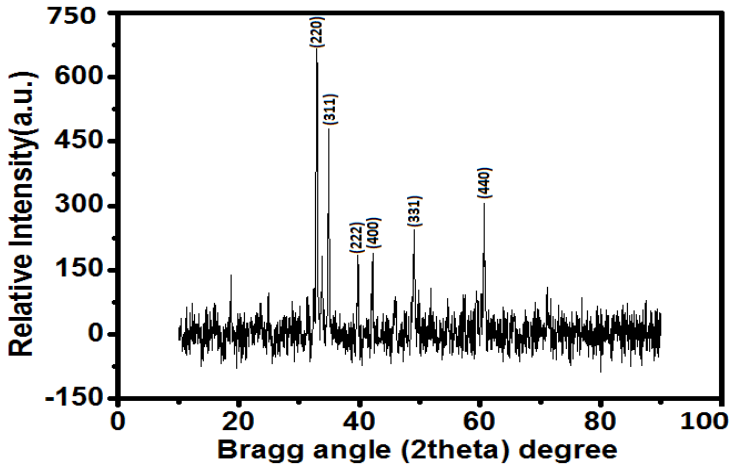

Figure 1. X-ray diffraction pattern and HRTEM image of $MgFe_2O_4$ MNP's

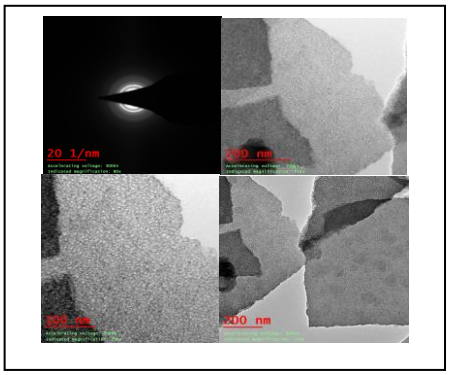

Figure 2. Powder XRD pattern for the $MgFe_2O_4$ nanoparticle

3.2 Synthesis of novel compounds (4-6)

The targeted novel 6,6'-(1,4-phenylene)bis(4-phenylpyrimidin-2-ol) **4**, 6,6'-(1,4-phenylene)bis(4-phenylpyrimidin-2-amine) **5** and (6,6'-(1,4-phenylene)bis(4-phenylpyrimidin-2-thiol) **6** were synthesized by treating chalcone (1mmol) **3,** bifunctional nucleophiles (2mmol) (like urea, guanidine nitrate and thiourea) under $MgFe_2O_4$ as catalyst (10mmol%) at refluxing ethanol condition for the appropriate time period. The $MgFe_2O_4$ heterogenous catalyst was easily removed by using simple external magnet. The products were obtained in 85-90% yields. The precursor **3** was prepared by reacting one mole of terphthaldehyde **1** with two moles of acetophenone **2** under Claisen-Schmidt condensation reaction condition (Scheme 1).

Scheme 1. Synthesis of ,6'-(1,4-phenylene)bis(4-phenylpyrimidin-2-ol/-2-amine/2-thiol (4-6)

3.3 Recovery and Reusability of catalyst

$MgFe_2O_4$ nano catalyst can very easily be recovered back from the reaction mixture by applying external magnetic field owing to the supermagnetic nature of Fe_3O_4 nanoparticles at room temperature. Reusability of magnetic ferrite nano particles were investigated by repetitive use of recovered catalyst. It was found that catalyst showed no appreciable change in activity even after five runs **(Fig. 2)**.

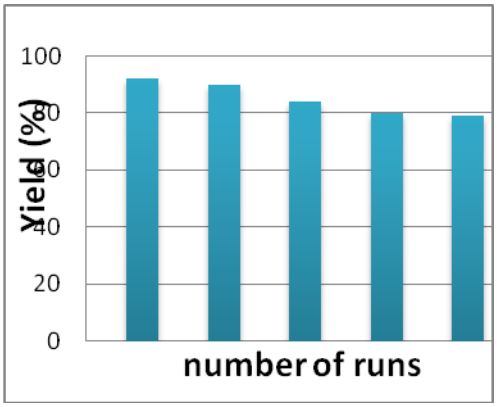

Fig 2. Reusability of catalyst for its catalytic activity

3.4 Antimicrobial screening

The antibacterial activity of newly synthesized compounds (4-6) was performed against *Bacillus subtilis* (MTCC No.121), *Escherichia coli* (MTCC No.118), *Staphylococcus aureus* (MTCC No. 96) and *Salmonella typhi* (MTCC No. 98) bacteria while antifungal activities was performed against *Aspergillus niger* (MTCC No. 281) and *Candida albicans* (MTCC No. 2479) using disc diffusion method table 1. Stock solutions of the synthesized compounds were prepared in the different concentrations, viz., 100µg/ml, 200µg/ml, 300µg/ml and 400µg/ml using dimethylsulfoxide (DMSO) as solvent for antimicrobial as well as antifungal activity. The results of all antibacterial and antifungal activity are summarized in table 1. The average diameter of the zone of inhibition in the range of 2.57-19.87 mm indicates significant antimicrobial activities. When compared with ciprofloxacin drug compound **5** showed comparable antibacterial activities while **4** and **6** showed moderate activities. The order of antibacterial activity among the compounds is **5**>**4**>**6.** Moreover the average zones of inhibition in the range of 1.80-6.50 indicates significant antifungal activity for the same compounds as compared to that of clotrimazole with inhibition zone (5.12-7.5 mm). Compound **4** showed high antifungal activity while compound **5** and **6** showed moderate activity. The order of antifungal activities among the compounds is **4**>**5**>**6**.

The minimum inhibition concentration (MIC) was also determined for all the newly synthesized compounds. The values of compounds **4-6** are depicted in Table 2. *S. aureus* showed the best MIC for compound **5** whereas compound **6** gave the lowest MIC. The study of cytotoxicity for the compounds **4-6** on living tissues is also under investigation results for these studies may be provided in our further reports.

Table 1. Antibacterial and antifungal activity result for compound (4-6)

Zone of inhibition ± S.D.									
Compounds	**Antibacterial activity**					**Compounds**	**Antifungal Activity**		
	Concn.	***B.subtilis***	***S.aureus***	***S.typhi***	***E.coli***		**Concn.**	***A.niger***	***C.albicans***
4	100µg/mL	3.12±0.47	3.37±0.62	2.87±0.47	3.62±0.85	**4**	100µg/mL	6.50±0.40	5.02±0.45
	200µg/mL	4.12±0.47	4.12±0.47	3.75±0.86	3.37±1.93		200µg/mL	8.75±0.28	6.15±0.78
	300µg/mL	4.62±0.85	5.0±0.57	2.87±0.47	3.87±0.47		300µg/mL	12.87±0.47	8.66±0.85
	400µg/mL	4.75±0.86	4.12±0.47	3.25±0.28	4.12±0.47		400µg/mL	14.12±0.47	11.02±0.87
5	100µg/mL	7.63±0.62	19.25±0.64	16.75±0.95	8.00±0.91	**5**	100µg/mL	3.37±0.62	1.80±0.12
	200µg/mL	9.62±2.17	19.87±0.75	19.87±0.47	8.75±0.64		200µg/mL	4.37±0.25	2.14±0.15
	300µg/mL	10.00±1.29	23.00±0.57	20.25±0.50	10.75±2.16		300µg/mL	5.40±0.27	4.89±0.79
	400µg/mL	14.80±1.31	21.12±0.75	21.00±0.57	14.62±1.88		400µg/mL	7.00±0.40	5.45±0.48
6	100µg/mL	3.25±0.50	2.57±0.47	3.25±0.50	3.00±0.57	**6**	100µg/mL	3.07±0.56	2.06±0.45
	200µg/mL	3.75±0.5	3.87±0.47	4.00±0.57	3.12±0.75		200µg/mL	4.37±0.47	3.89±0.87
	300µg/mL	3.87±0.47	4.87±0.47	4.87±0.47	3.37±0.25		300µg/mL	5.87±0.47	4.23±0.48
	400µg/mL	4.12±0.47	6.0±0.57	6.87±0.47	3.62±0.25		400µg/mL	6.37±0.62	6.12±0.45
Control(DMSO)*		***	***	***	***	**Control(DMSO)***		***	***
Ciprafloxacin[a]	100µg/mL	10.03±0.75	20.00±0.89	18.25±0.98	9.04±0.45	**Clotrimazole**[b]	100µg/mL	7.50±0.89	5.12±0.45

Note: Concn. of the compounds were 10mg/mL in DMSO, [a]Standard drug for antibacterial activity, [b]Standard drug for antifungal activity, [*]DMSO was taken as control

Table 2. Minimum Inhibition Concentration (MIC) of compounds (4-6)

	MIC (µg/mL) Compounds		
Bacteria	**4**	**5**	**6**
B.subtilis	1.92	3.89	1.80
S.aureus	1.79	9.82	1.35
S.typhi	1.69	8.42	1.69
E.coli	1.95	4.19	1.59

4. Conclusions

In summary we have efficiently developed a protocol for the synthesis of novel 6,6'-(1,4-phenylene)bis(4-phenylpyrimidin-2-ol) **4**, 6,6'-(1,4-phenylene)bis(4-phenylpyrimidin-2-amine) **5** and (6,6'-(1,4-phenylene)bis(4-phenylpyrimidin-2-thiol) **6** in very good yields by using magnesium ferrite nanoparticles as cheaper heterogeneous catalyst working in milder reaction conditions, shorter reaction time, simple and cleaner workup procedure. Catalyst can be reused atleast five times efficiently without losing its catalytic property. The antimicrobial activities of these compounds were examined and the results showed that compound **5** exhibited comparable antibacterial activity with that of ciprofloxacin. While compound 4 showed antifungal activities comparable to clotrimazole as standard drug. Biological potential of compounds **4** & **5** being as good as clotrimazole and ciprofloxacin respectively, can open plethora of opportunities in the field of research in antibiotics. Cytotoxicity for the compounds **4-6** is under investigation in our laboratory.

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