

SYNTHESIS AND ANTIFUNGAL ACTIVITY OF N (4-1H-BENZO [d] IMIDAZOLE -2YL) PHENYL-2-(4-HYDROXY-6-SUBSTITUTED PYRIMIDIN-2YL THIO /SULFONYL ACETAMIDE DERIVATIVES

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ABSTRACT

Synthesis of pyrimido-benzimidazole derivatives were in appreciable yield. The structures of title compounds were characterized by IR, ¹H NMR, and Mass spectral data. All of these derivatives were evaluated for their antifungal activity against *Candida albicans*, *Aspergillus Niger*, *Aspergillus flavus* and *Candida lunata* and showed good to moderate antifungal activity as compared to standard drug Nystatin.

KEY WORDS

Synthesis, pyrimidine, benzimidazole, antifungal activity.

INTRODUCTION

Nitrogen heterocycles often take part of immense importance as they are part of nucleic acids, vitamins, proteins and biologically molecular systems. Literature survey reveals that a large number of heterocyclic compounds containing pyrimidine moiety are majorly found to be attributed with different types of biological activities viz., insecticidal¹, antimicrobial², antiviral³ etc. Pyrimidines exhibit great importance in fundamental metabolisms⁴⁻⁶. Various analogues of thiopyrimidines such as 2-thiouracil and 2, 4-dithiouracil possess biological properties besides of their fundamental constituents of nucleic acids⁷⁻¹³. Benzimidazoles are known to be a group of biologically active compounds, possessing anti-viral, anti-helminthic, anti-fungal, anti-hypertensive and

anti-tumor activities¹⁴⁻¹⁶. Accordance to these observations, it has been considered to prepare new chemical entities that containing pyrimidine and benzimidazole moieties as important potential pharmacologically important molecules.

MATERIALS AND METHODS

Melting points were determined in open capillary tubes and are uncorrected. Progress of the reaction was monitored by TLC plates, ¹H NMR spectra were recorded on a Bruker 300 MHz instrument in DMSO/CDCl₃ using TMS as internal standard. Chemical shifts (δ) are expressed in ppm. IR spectra (KBr pellet) were recorded on a Perkin-Elmer BX series FT-IR spectrometer; elemental analyses were performed on a Perkin-Elmer 240 CHN analyzer.

Scheme:

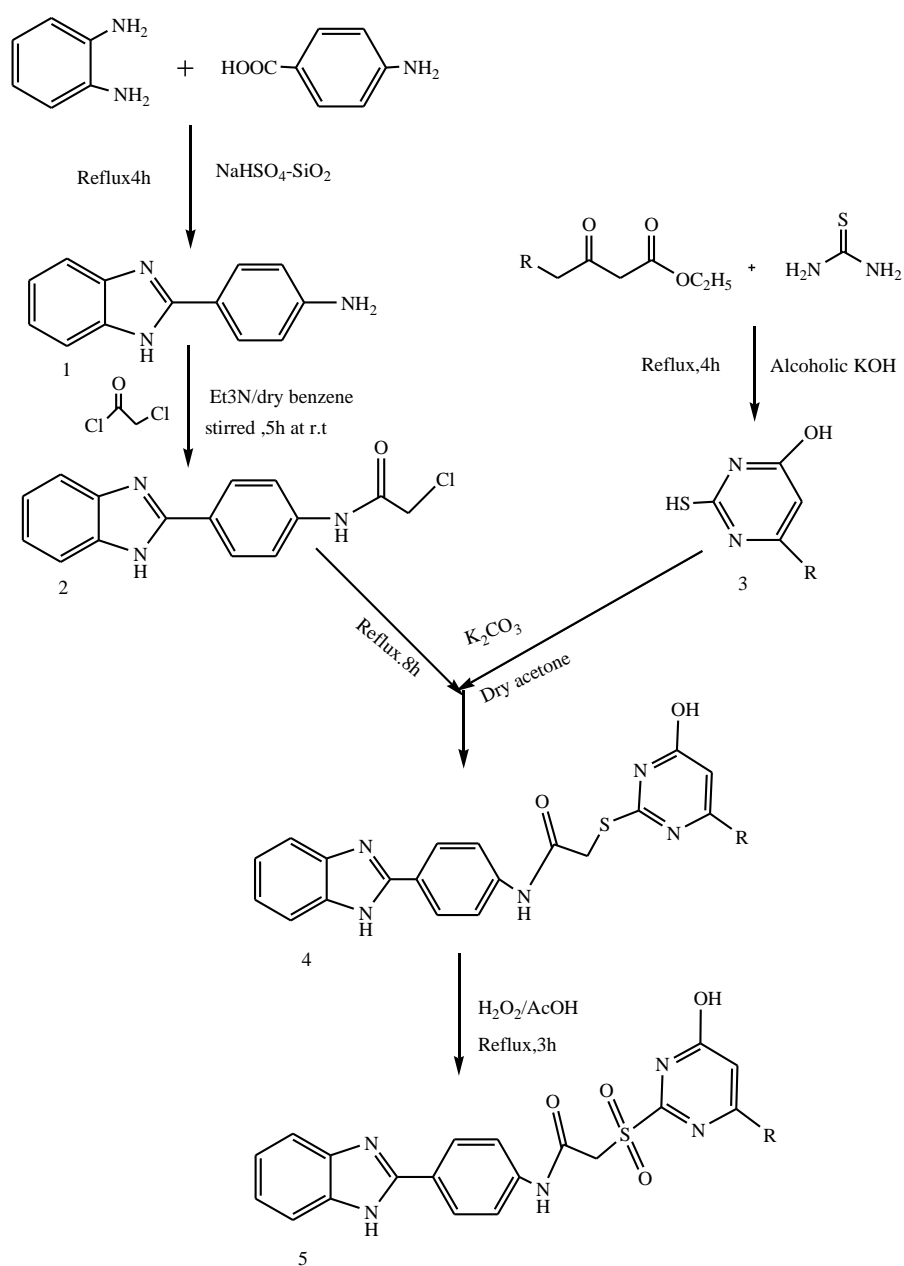


Table 1:

Compound	R	Compound	R
4a	-CH ₃	5a	-CH ₃
4b	-Ph	5b	-Ph
4c	-CF ₃	5c	-CF ₃
4d	-CH ₂ -OCH ₃	5d	-CH ₂ -OCH ₃
4e	2-C ₆ H ₄ Cl	5e	2-C ₆ H ₄ Cl

Synthesis of 4-(1H-benzo[d]imidazol-2-yl) aniline (1)

A mixture of p-amino benzoic acid (0.03 mmol) and o-phenylene diamine (0.03 mmol) and $\text{NaHSO}_4 \cdot \text{SiO}_2$ (25% wt.) in 10 mL of ethanol was heated under reflux at 180°C for 4 h. The reaction mixture was partially cooled, poured on to crushed ice and neutralized with 10% NaOH solution. The precipitated product was collected by vacuum filtration, washed with excess 10% NaOH solution was dried and recrystallized from ethanol.

Synthesis of N-(4-(1H-benzo[d]imidazol-2-yl) phenyl)-2-chloroacetamide (2)

In an ice bath, a solution of compound 1 (10 mmol) and triethylamine (0.5 ml) in dry benzene (10 ml) was stirred for 15 min 10 mmol of Chloroacetyl chloride was added and the reaction mixture was stirred for 4-5 hr. After completion of the reaction, the mixture was poured, with continuous stirring, on crushed ice. The solid formed was collected by vacuum filtration, washed with ethyl acetate, and recrystallized from ethanol.

Synthesis of 2-mercapto-6- substituted pyrimidin-4-ol (3)

A mixture of ethyl aceto acetate (substituted) (1mmol) and thio urea (1mmol) in alcoholic KOH (10 ml) was reflux for about 4 h, after completion of the reaction, the solid mixture was washed with cold water to remove the excess of thio urea and then filtered. The filtrate was concentrated and the solid product was recrystallized from ethyl acetate or ethanol. The remaining compounds were prepared by similar procedure with minor change in reaction conditions.

These compounds were purified by recrystallisation from suitable solvents. A similar method was used for the preparation of different derivatives (3a-e).

Synthesis of N-(4-1H-benzo (d) imidazole-2-yl) phenyl)-2-(4-hydroxy-6- methyl-pyrimidin 2yl thio) acetamide (4): Equimolar mixture of compound (2), compound (3) and K_2CO_3 (1mmol) in dry acetone (50 ml) was refluxed for 8 h. After cooling, solution was evaporated until dryness. The residue was washed with water and recrystallized from ethanol. The

remaining compounds were prepared by similar procedure with minor change in reaction conditions

(4a) IR: (KBr) $\lambda_{\text{max}}/\text{cm}^{-1}$: 3509 (-OH); 3313 (N-H, benzimidazole); 2942 (C-H, aliphatic); 1720 (C=O-NH); 1621 (C=N); 671 (C-S). ^1H NMR (DMSO d_6 , 300 MHz, δ ppm): 8.0 (s, NH, benzene); 7.65-7.75 (m, 2H, ArH); 7.46 (m, 2H, ArH); 7.26 (m, 2H, ArH); 6.32 (s, 1H-CH pyrimidine); 5.0 (s, -OH pyrimidine); 5.0 (s, NH, benzimidazole); 3.87 (s, 2H, CH_2 -S); 2.35 (s, 3H, - CH_3 pyrimidine). MS, m/z (%), 392 (M^+). Anal. Calcd. For $\text{C}_{20}\text{H}_{17}\text{N}_5\text{O}_2\text{S}$: C, 61.71; H, 4.38; N, 17.89; Found: C, 61.50; H, 4.02; N, 16.85.

(4b): IR: (KBr) $\lambda_{\text{max}}/\text{cm}^{-1}$: 3514 (-OH); 3325 (N-H, benzimidazole); 2942 (C-H, aliphatic); 1725 (C=O-NH); 1618 (C=N); 674 (C-S). ^1H NMR (DMSO d_6 , 300 MHz, δ ppm): 8.2 (s, NH, benzene); 7.68-7.72 (m, 4H, ArH); 7.46 (m, 2H, ArH); 7.34 (m, 2H, ArH); 7.20-7.27 (m, 3H, ArH); 6.3 (s, 1H-CH pyrimidine); 5.0 (s, -OH pyrimidine); 5.0 (s, NH, benzimidazole); 3.85 (s, 2H, CH_2 -S); MS, m/z (%), 454 (M^+). Anal. Calcd. For: $\text{C}_{25}\text{H}_{19}\text{N}_5\text{O}_2\text{S}$; C, 66.21; H, 4.22; N, 15.49%; Found: C, 66.02; H, 4.15; N, 15.25%.

(4c): IR (KBr) $\lambda_{\text{max}}/\text{cm}^{-1}$: 3506 (-OH); 3317 (N-H, benzimidazole); 2938 (C-H, aliphatic); 1721 (C=O-NH); 1615 (C=N); 668 (C-S). ^1H NMR (DMSO d_6 , 300 MHz, δ ppm): 8.6 (s, NH, benzene); 7.68-7.78 (m, 4H, ArH); 7.46 (m, 2H, ArH); 7.26 (m, 2H, ArH); 6.36 (s, 1H-CH pyrimidine); 5.0 (s, -OH pyrimidine); 5.0 (s, NH, benzimidazole); 3.89 (s, 2H, CH_2 -S); MS, m/z (%), 446 (M^+). Anal. Calcd. For: $\text{C}_{20}\text{H}_{14}\text{F}_3\text{N}_5\text{O}_2\text{S}$; C, 53.93; H, 3.17; N, 15.72%; Found: C, 53.02; H, 3.06; N, 15.25%.

(4d): IR (KBr) $\lambda_{\text{max}}/\text{cm}^{-1}$: 3510 (-OH); 3321 (N-H, benzimidazole); 2943 (C-H, aliphatic); 1732 (C=O-NH); 1608 (C=N); 665 (C-S). ^1H NMR (DMSO d_6 , 300 MHz, δ ppm): 8.1 (s, NH, benzene); 7.65-7.74 (m, 4H, ArH); 7.45 (m, 2H, ArH); 7.26 (m, 2H, ArH); 6.32 (s, 1H-CH pyrimidine); 5.0 (s, -OH pyrimidine); 5.0 (s, NH, benzimidazole); 3.86 (s, 2H, CH_2 -S); 4.64 (s, 2H, CH_2 -O); MS, m/z (%), 422 (M^+); Anal. Calcd. For: $\text{C}_{20}\text{H}_{17}\text{N}_5\text{O}_4\text{S}$; C, 56.53; H, 3.89; N, 16.32%; Found: C, 56.02; H, 3.32; N, 15.85%.

(4e)IR (KBr) $\lambda_{\max}/\text{cm}^{-1}$: 3510 (-OH); 3321 (N-H, benzimidazole); 2943 (C-H, aliphatic); 1732 (C=O-NH); 1608 (C=N); 665 (C-S). ¹HNMR (DMSO d_6 , 300 MHz, δ ppm) 8.3 (s, NH, benzene); 7.68-7.70 (m, 4H, ArH); 7.42-7.50 (m, 3H, ArH); 7.34 (m, 2H, ArH); 7.20-7.27 (m, 3H, ArH); 6.35 (s, 1H-CHpyrimidine); 5.0 (s, -OH pyrimidine); 5.0 (s, NH, benzimidazole); 3.85 (s, 2H, CH₂-S); MS, m/z (%), 488 (M⁺), 489 (M+²); Anal. Calcd. For: C₂₅H₁₈ClN₅O₂S; C, 61.53; H, 3.72; N, 14.32%; Found: C, 61.02; H, 3.32; N, 14.15%.

Synthesis of N-(4-1Hbenzo (d) imidazole-2-yl) phenyl)-2-(4-hydroxy-6 methyl-pyrimidin-4yl sulfonyl) acetamide (5):

An ice cold solution the compound 4 (1mmol) in glacial acetic acid (30 ml) was treated with 30% H₂O₂ (20 ml) in portions. The contents were allowed to attain laboratory temperature and then refluxed for 3h. The reaction mixture was cooled and acetic acid was removed in vacuo. The residual portion was cooled by filtration and was further purified by recrystallization using water. The remaining compounds were prepared by similar procedure with minor change in reaction conditions.

(105a):IR (KBr) $\lambda_{\max}/\text{cm}^{-1}$: 3496 (-OH); 3358 (N-H, benzimidazole); 2885 (C-H, aliphatic); 1705 (C=O-NH); 1631 (C=N); 1168 (-SO₂); 681 (C-S). ¹HNMR (DMSO d_6 , 300 MHz, δ ppm): 8.2 (s, NH, benzene); 7.74 (m, 2H, ArH); 7.60-7.40 (m, 4H, ArH); 7.28 (m, 2H, ArH); 6.36 (s, 1H, ArH, pyrimidine); 6.0 (s, 1H, -OH-pyrimidine); 5.0 (s, -OH, pyrimidine); 5.0 (s, NH, benzimidazole); 4.35 (s, 2H, SO₂-CH₂); 2.39 (s, 3H, -CH₃, pyrimidine). MS, m/z (%), 423 (M⁺). Anal. Calcd. For: C₂₆H₂₀ClN₅O₂S; C, 56.74; H, 4.02; N, 16.52%; Found: C, 56.68; H, 4.01; N, 16.48%.

(105b)IR (KBr) $\lambda_{\max}/\text{cm}^{-1}$: 3510 (-OH); 3319 (N-H, benzimidazole); 1727 (C=ONH); 1620 (C=N); 1114 (-SO₂); 672 (C-S). ¹HNMR (DMSO d_6 , 300 MHz, δ ppm): 8.0 (s, 1H, NH, benzene); 7.72 (m, 2H, ArH); 7.50 (m, 2H, ArH); 7.38-7.24 (m, 7H, ArH); 6.42 (s, 1H, CH, pyrimidine); 5.66 (s, 1H, OH, pyrimidine); 5.0 (s, 1H, NH, benzimidazole); 4.42 (s, 2H, SO₂-CH₂). MS m/z (%) 485 (M⁺). Anal. Calcd. For: C₂₅H₁₉N₅O₄S; C, 61.85; H, 3.91; N, 14.43%; Found: C, 61.83; H, 3.89; N, 14.42%.

(105c)IR (KBr) $\lambda_{\max}/\text{cm}^{-1}$: 3503 (-OH); 3356 (N-H, benzimidazole); 1710 (C=ONH); 1628 (C=N); 1114 (-SO₂); 682 (C-S). ¹HNMR (DMSO d_6 , 300 MHz, δ ppm): 8.4 (NH, benzene); 7.70 (m, 4H, ArH); 7.607.40 (m, 4H, ArH); 7.28 (m, 2H, ArH); 6.36 (s, 1H, ArHpyrimidine); 6.0 (s, 1H, OHpyrimidin); 5.0 (s, OHpyrimidine); 5.0 (s, NH, benzimidazole); 4.35 (s, 2H, SO₂CH₂); MS, m/z (%), 477 (M⁺). Anal. Calcd. For: C₂₀H₁₄N₅O₄SF₃; C, 50.32; H, 2.92; N, 14.67%; Found: C, 50.62; H, 2.95; N, 14.72%.

(105d)IR (KBr) $\lambda_{\max}/\text{cm}^{-1}$: 3508 (-OH); 3349 (N-H, benzimidazole); 1717 (C=ONH); 1636 (C=N); 1119 (-SO₂); 672 (C-S). ¹HNMR (DMSO d_6 , 300 MHz, δ ppm) 8.2 (s NH, benzene); 7.74 (m, 4H, ArH); 7.45 (m, 2H, ArH); 7.28 (m, 2H, ArH); 6.83 (s, 1H, ArH pyrimidine); 6.2 (s, 1H, -OH pyrimidine); 5.1 (s, -OH pyrimidine); 5.0 (s, NH, benzimidazole); 4.35 (s, 2H, SO₂-CH₂); 4.60 (s, 2H, CH₂, pyrimidine); Anal. Calcd. For: MS, m/z (%), 454 (M⁺). C₂₁H₁₉N₅O₅S; C, 55.62; H, 4.22; N, 15.44%; Found: C, 50.61; H, 4.17; N, 15.45%.

(105e)IR (KBr) $\lambda_{\max}/\text{cm}^{-1}$: 3503 (-OH); 3329 (N-H, benzimidazole); 1708 (C=ONH); 1648 (C=N); 1123 (-SO₂); 676 (C-S). ¹HNMR (DMSO d_6 , 300 MHz, δ ppm): 8.5 (s NH, benzene); 7.72 (m, 4H, ArH); 7.33-7.45 (m, 4H, ArH); 7.16-7.26 (m, 4H, ArH); 7.0 (s, 1H, ArH pyrimidine); 6.4 (s, 1H, -OH pyrimidine); 5.0 (s, -OH pyrimidine); 5.0 (s, NH, benzimidazole); 4.38 (s, 2H, SO₂-CH₂); MS, m/z (%), 520 (M⁺). 522 (M+²) Anal. Calcd. For: MS, m/z (%), 454 (M⁺). C₂₅H₁₈N₅O₄ClS; C, 57.76; H, 3.49; N, 13.46%; Found: C, 57.81; H, 3.42; N, 13.48%.

Antifungal activity

The following fungal species are used to assess the antifungal activity of compounds, they are *Candida albicans* MTCC 227, *Aspergillus niger* MTCC 282, *Aspergillus clavatus* MTCC 1323, *Candida lunata* MTCC 226, these were obtained from Fungal Culture Collection Laboratory, Department of Microbiology, Kakatiya University, Warangal, Telangana.

Media for fungal cultures

Sabourad's Dextrose Agar Medium (SDA)

Peptone 10.00 g

Dextrose 40.00 g

Agar 20.00 g, Distilled water 1000 mL

pH 6.8

Assay

The antifungal activity of synthesized compounds was determined by agar well diffusion method¹⁷. The culture plates inoculated with test organisms were allowed to solidify and punched with sterile cork borer (7.0 mm diameter) to make open wells. The open wells were filled with 0.05 mL or 50 µL of the test compounds. The test was carried out on SDA Plates and incubated at 30°C and 22°C, respectively for 72 hrs. The test organisms were sub-cultured using potato-dextrose-agar medium. The tubes containing sterilized medium were inoculated with test fungi and after incubation at 37 °C for 48 hours, they were stored at 4 °C in refrigerator. The zones of inhibition were measured and recorded.

Antifungal assay

Antifungal activity of the synthesized compounds resulted significant activities of all the compounds tested. Activity was observed in dose dependent manner. Among the compounds screened derivatives of compound **5(a-e)** showed highest inhibition zones among the tested organisms. Highest zone of inhibition 14 was noticed with compound **4c,5c** compared to other compounds. *Aspergillus niger* was the most susceptible species towards the compounds tested. On the other hand, *Candida albicans* also showed susceptibility for the compounds screened. However, derivatives of compound (**4**) showed lesser activity compared to the derivatives of compound (**5**). The highest zones of inhibition showed by derivatives are compared with known standard Nystatin at 10 mg/mL (Table 2).

Table 2: Antifungal activity of compounds tested against various fungal pathogens.

Compound	Conc.	Zone of inhibition in mm			
		C.albicans	A.Niger	A.Flavus	C.Lunata
104a	10	8	8	4	5
104b	10	3	4	3	4
104c	10	10	12	10	9
104d	10	5	7	4	4
104e	10	6	8	6	7
105a	10	8	9	5	6
105b	10	4	5	4	5
105c	10	11	14	11	10
105d	10	6	8	5	5
105e	10	9	10	8	8
Standard		14	17	18	15

RESULTS AND DISCUSSION

Current study reports the devoted towards the research and development of highly efficient heterocyclic molecules with therapeutic potential. Efforts of our studies includes the synthesis of pyrimido benzimidazoles derivatives (**4&5**) synthesized by different substituent basing on pyrimidine ring. The starting material 2-(4-amino phenyl) benzimidazole is synthesized by using NaHSO₄-SiO₂, it is a heterogeneous and eco friendly catalyst¹⁸ the target compounds were synthesized according to procedure.

The data reveal that the compounds 4c, 5c have excellent antifungal activity against the test fungi and nearly equal to the standard drug. Compounds 4e, 5e have showed moderate to activity where as the remaining showed least activity. The compounds (**5**) showed better activity than the compounds (**4**), because sulfonyl group is more active than the thio group.

CONCLUSION

The present study reports successful synthesis of compounds **4 (a-e)** and **5 (a-e)** in good yield and these compounds exhibit moderate anti-fungal activity.

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REFERENCES

1. M S. Shingare; *Indian J Chem*, 1983, 22B, 714.
2. M M.Ghorab and S G.Abdel; *Indian J Heterocycl. Chem*, 1994, 4, 103.
3. E.Wagner, L.Be can and E.Nowakowska; *Bioorg. Med. Chem*, 2004, 12,265.
4. Z. Wang and T M. Rana; *Biochemistry*, 1996, 35, 6491.
5. R P.Martin, JM. Scheller, A J C. Stahl and G. Dirheimer; *Biochem. Biophys. Res. Commun*, 1976, 70, 997.
6. M. Altweg and E. Kubli; *Nucleic Acids Res*, 1980, 8, 215.
7. G A. Jeffrey and W. Saenger; *Springer-Verlag*, New York, 1991.
8. H.Charbonneau N. Walsh, and J A. Be; *Proc Natl. Acad. Sci., U.S.A*, 1986, 83, 9308.
9. S. Topiol; *Trends Biochem. Sci*, 1987, 12, 419.
10. C J. Hunter, D.F F. Deen and L J. Marton; *Internat J. Cancer*, 1989, 44, 658.
11. R. Pieters, D R. Huismans, A H. Loonen, K. Hahlen and A J. Veerm; *Jpn. J. Cancer Res*, 1991, 82, 1051.
12. L M. Beauchamp L. Serling, J.E Kesley, K K. Biron, P.Collins, J. Selway, JC.Lin and H. Schaeffer; *J. Med. Chem*, 1988, 31, 144. R.Pieters,D R.Huismans,A H.Loonen,G J. Peters, K.Hahlen, A.Vander Does-van den Berg, E.R. VanWeiring and A J P .Veerman; *Internat J. Cancer*. 1992, 51, 213.
13. JS.Kim, Gatto, C. Yu, A. Liu, L.F and E.J LaVoice; *J.Med. Chem*, 1996, 39, 992.
14. N. Kohei and N.Takeheiko; *J. Med. Chem*, 1993, 36, 2182.
15. J. Lu, B.Yang, Y, Y. Bai; *Synth. Commun*, 2002, 32, 3703.
16. S. Magaldi, S. Mata-Essayag, C. H. D. Capiles, C. Perez, M. Colella, C. Olaizola and Y. Ontiveros, Well Diffusion for Antifungal Susceptibility Testing, *Int. J. InfectiousDiseases*, 2004, 8, 39.
17. Atul Chaskar, Vikas Padalkar, Kiran Phatangare, Santosh Takale and Kaliyappan Murugan: *Green Chemistry Letters and Reviews*, 2013, 6,217.



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