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Synthesis and antimicrobial activity of novel C-linked imidazole glycoconjugates

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Abstract—Novel C-linked imidazole derivatives have been synthesized in good to excellent yields and characterized by analytical and spectral analysis. Four of the newly synthesized compounds exhibited moderate antibacterial activity.

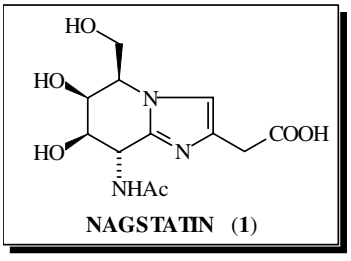
The prevalence of imidazoles in natural products and pharmacologically active compounds has instituted a diverse array of synthetic approaches to these heterocycles.[1] Sugar annulated imidazoles have received particular interest due to the remarkable capacity of naturally occurring nagstatin (Fig. 1) to inhibit *glucosaminidases*.[2] In 1992 Aoyagi, Aoyama and their coworkers published the structure of the natural product nagstatin and showed that this imidazolo sugar is a very potent inhibitor of some *glucosaminidases*, for example, with a K_i value of 4 nM for the *N*-acetyl-β-D-glucosaminidase of bovine kidney enzyme.[2]

The discovery of nagstatin proved to be of interest for the defined deciphering of glycosidase-catalyzed hydrolysis of polysaccharides by way of the so-called lateral protonation mechanism.[3–5]

The importance of sugar annulated imidazoles has generated an interest in us to explore a route leading to a collection of hitherto scarcely investigated C-glycosyl tetra substituted imidazoles. Aside from simple expectation that the sugar residues having hydroxyl groups in the imidazole moiety should increase the water solubility and bioavailability, other interesting biological properties may arise from glycosylation considering the extensive and essential role of carbohydrate molecules in the complex machinery of various glycoconjugate biological activities.[6] Glycosylation of heterocyclic compounds that are rich in biological activity is a field of increasing interest, for example, synthesis of various guanidinoglycosides displays improved biological properties (anti-inflammatory and anti-HIV activities) with respect to the non-glycosylated guanidino derivatives.[7]

Recently Sharma et al.,[8] reported an efficient synthesis of tri substituted glycosyl imidazoles in the presence of $ZrCl_4$. Though few methods are available for tri substituted glycosyl imidazoles, there appear no reports for the synthesis of C-2 glycosyl tetra substituted imidazoles. This fact has rekindled an increased interest in us to obtain C-glycosyl tetra substituted imidazoles.

In continuation of our work for the synthesis of tetra-substituted imidazoles,[9] we now aim at the synthesis and collection of monoglycosylated tetra substituted



NAGSTATIN (1)

Figure 1.

Keywords: Bioactive; C-2 chiral polyhydroxy imidazoles; Potassium dodecatungstocobaltate trihydrate.
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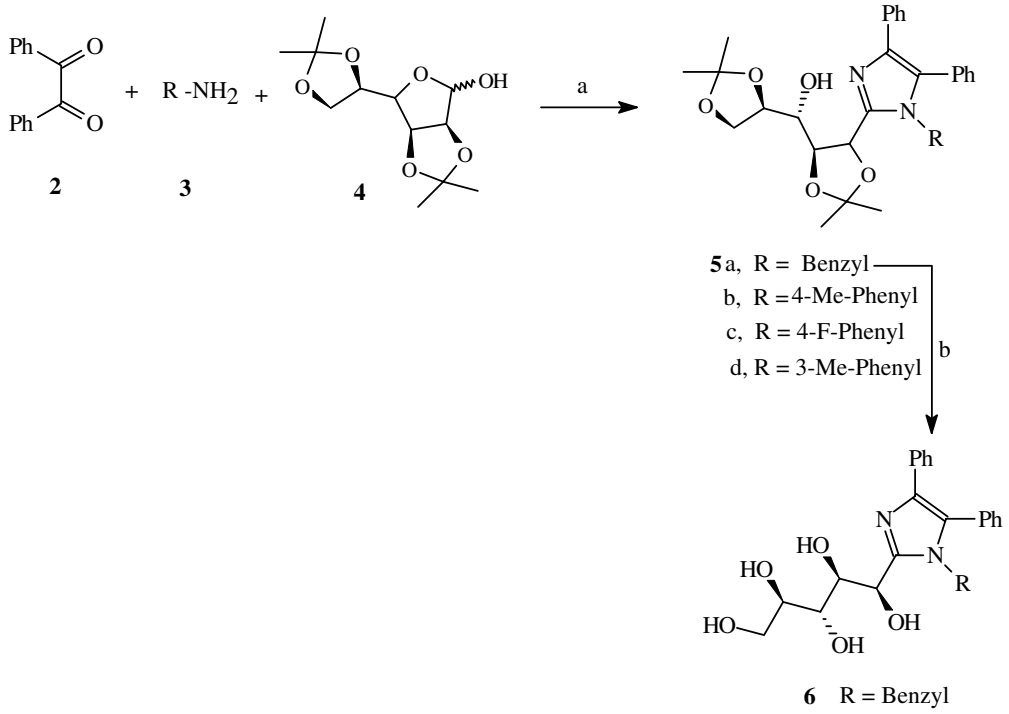
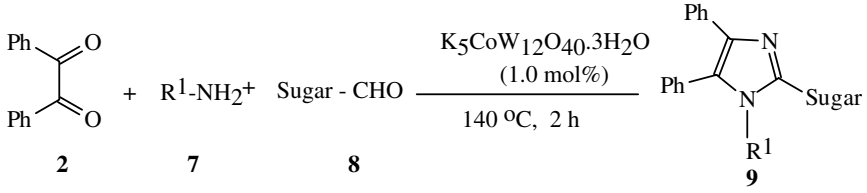


Scheme 1. Reagents and conditions: (a) $K_5C_0W_{12}O_{40}{\cdot}3H_2O$ + NH_4OAc (1.6 equiv), 140 °C, 2 h; (b) DOWEX 50WX8 (H^+), EtOH/H_2O (1:1), 60 °C, 3 h.



Scheme 2.

imidazole scaffolds via parallel synthesis employing suitable carbohydrate component as one of the reactants. Thus, a new and novel method for the synthesis of tetra substituted imidazole derivatives with glycosyl moiety at C-2 position has been developed using appropriate sugar aldehydes, benzil, aromatic amines and ammonium acetate by using potassium dodecatungstocobaltate trihydrate (PDTC) (1.0 mol%) as a heterogeneous catalyst under solvent-free conditions at 140 °C (Scheme 1) in 60.0–68.4% yields. All the nine compounds were screened for their antibacterial and antifungal activities.

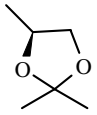
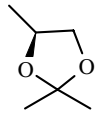
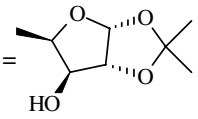
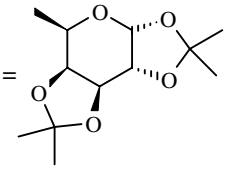
The four-component condensation of benzil (**2**), amine (**3a–d**), mannose diacetonide (**4**) and ammonium acetate in the presence of 1.0 mol% of $K_5CoW_{12}O_{40}{\cdot}3H_2O$ at 140 °C for 2 h gave mannosyl imidazoles (**5a–d**). The spectral data of compound **5a** were in agreement with the proposed structure. Corresponding C-2 chiral polyhydroxy imidazole (**6**) was obtained (71.52%) as colourless solid from **5a** by cleavage of acetal with Dowex® (50WX8-H^+) in EtOH: H_2O (1:1) at 60 °C (Scheme 1).

Under the same conditions, this approach can be repeated for the synthesis of hitherto unreported tetra-substituted novel (C-2 chiral) imidazolyl sugars (**9a–d**) using benzil, various sugar aldehydes, amines, ammonium acetate and $K_5CoW_{12}O_{40}{\cdot}3H_2O$ (Scheme 2) (Table 1). The structures of compounds **5a–d**, **6**, and **9a–d** were deduced from ^1H and ^{13}C NMR, IR, mass spectral data and elemental analysis.[10] It was found that in the absence of PDTC there was no progress in the four-component condensation reaction.

All the newly synthesized compounds **5a–d**, **6**, and **9a–d** were screened for antibacterial and antifungal activities.[11] All the compounds **5a–d**, **6**, and **9a–d** showed activity against Gram-negative and Gram-positive bacteria. Compounds **5c**, **5d**, **9c**, and **9d** showed moderate antibacterial activity against *Pseudomonas aeruginosa*. Compound **9d** showed more activity towards Gram-positive bacteria (i.e., *Bacillus subtilis*) (Table 2).

All the compounds were screened for antifungal activity against *Saccharomyces cerevisiae*, *Aspergillus niger*, *Rhizopus oryzae*, and *Candida albicans* by agar cup diffusion method[12] using Amphotericin-B as standard. However, none of the compounds showed antifungal activity.

Table 1.

Entry	R′-NH2 (**7a–d**)	Sugar	Time (h)	$[\alpha]_D^{20}$	Yield (%)[a]
a	4-F-phenyl	**9a** = 	2	−38.6	60
b	4-Methyl-phenyl	**9b** = 	2	−42.8	65
c	Benzyl	**9c** = 	2	−64.0	55
d	4-Methyl-phenyl	**9d** = 	2	−18.8	63

[a] Isolated yield.

Table 2. Antibacterial activities as MIC (150 μg/mL) for **5a–d**, **6** and **9a–d**

Compound	Gram positive organisms			Gram negative organisms		
	B. subtilis	*S. aureus*	*S. epidermidis*	*E. coli*	*P. aeroginosa*	*K. pneumoniae*
5a	75	75	150	75	37.5	75
5b	75	75	150	75	75	75
5c	150	150	150	75	75	75
5d	150	150	75	75	37.5	150
6	75	75	75	75	75	75
9a	75	75	75	37.5	37.5	75
9b	150	150	75	75	150	75
9c	150	150	150	150	37.5	75
9d	37.5	37.5	75	75	75	75
Streptomycin	6.25	1.562	1.562	2.35	3.125	3.125
Penicillin	1.526	6.25	3.125	7.81	12.5	6.25

In conclusion, C-2 chiral tetra substituted imidazoles were synthesized, characterized and screened for antibacterial and antifungal activities. Compounds **5c**, **5d**, **9c**, and **9d** showed moderate antibacterial activity. Though the compounds showed no antifungal activity, the promising results in antibacterial activity would give scope for further work in this area.

Acknowledgments

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References and notes

1. (a) Grammitt, M. R.; Katrizky, A. R.; Boulton, A. J.. In *Advances in Heterocyclic Chemistry*; Academic: New York, 1989; p 241; (b) Grammitt, M. R.; Katrizky, A. R.; Boulton, A. J.. In *Advances in Heterocyclic Chemistry*; Academic: New York, 1970; Vol. 12, p 103.
2. (a) Aoyagi, T.; Suda, H.; Uotani, K.; Kojima, F.; Aoyama, T.; Horiguchi, K.; Hamada, M.; Takeuchi, T. *J. Antibiot.* **1992**, *45*, 1404; (b) Aoyama, T.; Naganawa, N. H.; Suda, H.; Uotani, K.; Aoyagi, T.; Takeuchi, T. *J. Antibiot.* **1992**, *45*, 1557.
3. Heightman, H.; Vasella, T. *Angew. Chem. Int. Ed.* **1999**, *38*, 750.
4. Tatsuta, K.; Miura, S.; Ohta, S.; Gunji, H. *J. Antibiot.* **1995**, *48*, 286.
5. Sinnott, M. L. *Chem. Rev* **1990**, 1171.
6. (a)*Essentials of Glycobiology*; Varki, A., Cummings, R., Esko, J., Freeze, H., Hart, G., Marth, J., Eds.; Cold Spring Harbor Laboratory Press: New York, 1993; (b) Varki, A. *Glycobiology* **1993**, *3*, 97.
7. Lin, P.; Lee, C. L.; Sim, M. M. *J. Org. Chem.* **2001**, *66*, 8243.
8. Sharma, G. V. M.; Jyothi, Y.; Sree Lakshmi, P. *Synth. Commun.* **2006**, *36*, 2991.

9. (a) Nagarapu, L.; Satyender, A.; Srinivas, K. *J. Mol. Catal. A: Chem.* **2006**, *266*, 104; (b) Srinivas, K.; Srinivasu, V. N. V.; Dhanraj, B. O.; Nagarapu, L. *J. Mol. Catal. A: Chem.* **2006**, *266*, 109.

10. Experimental: Melting points were measured with Fiescher–Johns melting point apparatus and are not corrected. ^{1}H NMR spectra were recorded with an AVANCE 300 Bruker at 300 MHz and Gemini 200 MHz in $CDCl_3$. Chemical shifts relative to TMS as internal standard are given as δ values in ppm. ^{13}C NMR spectra were recorded in $CDCl_3$and in DMSO-d_6 on a Varian (75 MHz) spectrometer. IR spectra were taken with a Perkin-Elmer 1725Λ FT-IR spectrophotometer. EI-MS mass spectra were measured at 70 eV (EI).

General procedure for C-2 mannosyl tetra substituted imidazoles (**5a–d**) and C-2 glycosyl tetra substituted imidazoles (**9a–d**): To a mixture of benzil (2.0 mmol), amine (2.0 mmol), mannose diacetonide (2.0 mmol) or sugar aldehyde (2.0 mmol), ammonium acetate (3.2 mmol) and $K_5CoW_{12}O_{40}.3H_2O$ (64 mg, 1.0 mol%) were mixed well and heated at 140 °C for 2 h. The reaction mixture was cooled to room temperature, added acetone (10 mL), filtered (to remove catalyst), filtrate was concentrated under reduced pressure and syrupy residue was purified by column chromatography using EtOAc: hexane (1:9) as eluent to obtain pure compounds.

Compound **5a**. Colourless powder, Yield: 61%, m.p. 124–126 °C, $[\alpha]_D^{20}$ −8.1 (*c* 1, $CHCl_3$), IR (KBr): υ 3331, 3053, 2982, 2930, 1890, 1515, 1382^{-1}, ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.31, 1.41, 1.46 (3s, 12H, 4× –CH_3), 3.67 (brs, 1H, –OH), 4.02 (m, 3H, 5′-H_a, 5′-H_b, 3′-H), 4.98 (d, 1H, 2′-H, *J* = 4.6 Hz), 5.07 (dd, 2H, –CH_2Ph), 5.16 (dd, 1H, 4′-H, *J*=2.3), 5.26 (d, 1H, 1′-H, *J* = 3.9 Hz), 6.84–7.43 (m, 15H, Ar-H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 25.61, 29.14, 47.53, 66.76, 70.09, 71.40, 77.67, 109.42, 119.43, 126.53, 128.23, 129.09, 131.21, 137.31, 144.03. ESI: *m*/*z* = 541 [M^++H]. Anal. Calcd for $C_{33}H_{36}N_2O_5$: C, 73.31; H, 6.71. Found: C, 73.38; H, 6.82.

Compound **5b**. Colourless powder, Yield: 68.4%, m.p. 162–164 °C, $[\alpha]_D^{20}$ −72.2° (*c* 1, $CHCl_3$), IR (KBr): υ 3454, 2361, 1384^{-1}, ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.30,1.38, 1.55 (3s, 12H, 4× –CH_3), 2.37 (s, 3H, Ar-CH_3), 3.55 (d, 1H, 4′-H, *J* = 3.1 Hz), 3.95–4.09 (m, 3H, 5′-H_a, 5′-H_b, 3′-H), 4.72 (d, 1H,1′-H, *J* = 7.8 Hz), 5.25 (dd, 1H, 2′-H, *J* = 3.1, 6.1 Hz), 7.06–7.50 (m, 14H, Ar-H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 21.06, 25.33, 26.76, 65.50, 70.14, 76.54, 109.16, 126.43, 127.28, 129.39, 130.73, 138.32, 143.78. ESI: *m*/*z* = 541 [M^+ + H]. Anal. Calcd for $C_{33}H_{36}N_2O_5$: C, 73.31; H, 6.71. Found: C, 73.29; H, 6.74.

Compound **5c**. Colourless powder, Yield: 65.4%, m.p. 120–122 °C, IR (KBr): υ 3447, 2925, 1880, 1522, 1372^{-1}, ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.21, 1.26, 1.32 (3s, 12H, 4× –CH_3), 3.5 (br s, 1H, -OH), 4.0 (m, 4H, 5′-H_a, 5′-H_b, 3′-H, 2′-H), 4.7 (d, 1H, 1′-H, *J* = 8.3 Hz), 5.2 (dd, 1H, 4′-H, *J* = 2.8 Hz), 7.0–7.4 (m, 14H, Ar-H). ESI: *m*/*z* = 545 [M^++H]. Anal. Calcd for $C_{32}H_{33}N_2O_5F$: C, 70.57; H, 6.10. Found: C, 70.55; H, 6.06.

Compound **5d**. Colourless powder, Yield: 67%, m.p. 145–147 °C, IR (KBr): υ 3444, 3043, 2924, 1888, 1511, 1385^{-1}, ^{1}H NMR ($CDCl_3$, 300 MHz): δ1.22, 1.31, 1.38, 1.54 (4s, 12H, 4× –CH_3), 2.3 (s, 3H, Ar-CH_3), 3.5 (br s, 1H, –OH), 3.9 (m, 4H, 5′-H_a, 5′-H_b, 3′-H, 2′-H), 4.7 (d, 1H, 1′-H, *J* = 8.3 Hz), 5.1 (dd, 1H, 4′-H, *J* = 3.0 Hz), 7.0–7.4 (m, 14H, Ar-H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 21.10, 25.33, 26.76, 26.93, 29.64, 66.52, 70.11, 70.69, 78.57, 109.18, 110.14, 125.14, 126.48, 127.85, 128.02, 129.13, 130.70, 135.39, 138.73, 143.56. ESI: *m*/*z* = 541 [M^+ + H]. Anal. Calcd. for $C_{33}H_{36}N_2O_5$: C, 73.31; H, 6.71. Found: C, 73.29; H, 6.73.

Synthesis of imidazolo C-2 chiral pentahydroxy compound (**6**). A stirred solution of **5a** (1.8 g, 3.57 mmol) in ethanol (40 mL) and water (40 mL), containing Dowex® (50WX8-H^+) resin (1.0 g), was heated to 60 °C for 3 h. The reaction mixture was cooled to room temperature and filtered; the resin was washed with ether (2 × 60 mL) and extracted with MeOH-aq.NH_3 (2 × 200 mL). The combined extracts were concentrated to give **6** (1.08 g, 71.52 %) as a colourless powder, m.p. 210–212 °C, $[\alpha]_D^{20}$ −4.1 (*c* 1, MeOH), IR (KBr): υ3447, 1643, 1383, 1206, 1140, 1038^{-1}, ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.62 (m, 3H, 5′-Ha, 5′-Hb & 3′-H), 3.82 (d, 1H, 4′-H, *J* = 6.7 Hz), 4.30–4.40 (t × d, 2H, -CH_2Ph, *J* = 4.5 Hz), 4.86 (t, 1H, 2′-H, *J* = 6.0 Hz), 5.31 (d, 1H, 1′-H, *J* = 6.0 Hz), 4.07, 5.72 (2 br s, 2 × OH), 5.08, 5.11, 5.24, (3s, 3 × OH), 6.89-7.64(m, 15H, Ar-H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 22.50, 28.43, 29.89, 31.68, 39.44, 46.83, 63.55, 67.14, 70.34, 71.07, 71.71, 79.18, 126.05, 126.34, 127.23, 128.12, 128.36, 128.80, 128.96. ESI: *m*/*z* = 461 [M^++H]. Anal. Calcd for $C_{27}H_{28}N_2O_5$: C, 70.42; H, 6.13. Found: C, 70.46; H, 6.16.

Compound **9a**. Syrup, Yield: 60%, $[\alpha]_D^{20}$ −38.6 (*c* 0.4, $CHCl_3$), IR (KBr): υ 1653, 1602, 1508, 1445,1376^{-1},^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.33, 1.45 (2 s, 2 × CH_3), 4.20 (t, 1H, 1′-H, *J* = 6.2 Hz), 4.73 (m, 2H, 2′a-H, 2′b-H), 7.00–7.48 (m, 14H, Ar-H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 25.99, 26.22, 67.63, 69.77,110.15, 115.64, 126.58, 127.20, 129.93, 130.73, 131.68, 134.23, 137.70, 144.46, 163.78. ESI: *m*/*z* = 415 [M^++H]. Anal. Calcd for $C_{26}H_{23}N_2O_2$: C, 75.34; H, 5.59. Found: C, 74.98; H, 6.62.

Compound **9b**. Syrup, Yield: 65%, $[\alpha]_D^{20}$−42.8 (*c* 0.5, $CHCl_3$), IR (KBr): υ 1666, 1603, 1512, 1446,1374^{-1}, ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.35, 1.50 (2 s, 2 × CH_3), 2.35 (s, 3H, Ar-CH_3), 4.18 (t, 1H, 1′-H, *J* = 6.0 Hz), 4.73 (m, 2H, 2′a-H, 2′b-H), 7.05–7.24 (m, 14H, Ar-H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 21.06, 26.08, 26.28, 67.85, 69.79,110.08, 120.29, 126.44, 127.29, 128.65, 129.43, 129.87, 130.51, 130.78, 133.08, 134.49, 137.69, 138.42, 144.43. ESI: *m*/*z* = 411 [M^++H]. Anal. Calcd for $C_{27}H_{26}N_2O_2$: C, 79.00; H, 6.38. Found: C, 79.18; H, 6.42.

Compound **9c**. Colourless powder, Yield: 60.8%, m.p. 115–117 °C, $[\alpha]_D^{20}$−64.1 (*c* 0.6, $CHCl_3$), IR (KBr): υ 3479, 2924, 1601, 1446, 1380, 1211^{-1}, ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.30 (2s, 6H, 2 × CH_3), 4.52 (d, 1H, 4′-H, *J* = 1.7 Hz), 4.61 (d, 1H, 3′-H, *J* = 4.2 Hz), 4.73 (d, 1H, 2′-H, *J* = 2.5 Hz), 5.0 (d, 1H, –CH_2Ph, *J* = 2.5 Hz), 5.3 (t, 1H, –CH_2Ph, *J* = 5.1 Hz.), 6.01 (d, 1H, 1′-H, *J* = 3.4 Hz), 6.5 (s, 1H, –OH), 6.99–7.39 (m, 15H, Ar-H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 26.71, 29.62, 47.30, 72.44, 84.52, 105.88, 111.66, 126.75, 128.18, 128.59, 128.88, 129.14, 130.04, 130.13, 130.91, 131.01, 131.09, 133.50, 136.18, 136.27, 136.35, 136.44,136.47, 143.25. ESI: *m*/*z* = 469 [M^++H]. Anal. Calcd for $C_{29}H_{28}N_2O_4$: C, 74.34; H, 6.02. Found: C, 73.98; H, 6.12.

Compound **9d**. Syrup, Yield: 60.8%, $[\alpha]_D^{20}$ −18.8 (*c* 0.45, $CHCl_3$), IR (KBr): υ 3446, 2925, 1741, 1668, 1604, 1447, 1376, 1258, 1071^{-1}, ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.32, 1.43, 1.49 (3s, 12H, 4 × CH_3), 2.06 (s, 3H, Ar-CH_3), 3.93–4.27 (m, 3H, 2′-H, 4′-H, 5′-H), 4.57 (dd, 1H, 3′-H, *J* = 6.7 Hz), 5.46 (d, 1H, 1′-H, *J* = 4.5 Hz), 6.5 (s, 1H, –OH), 7.31–7.50 (m, 14H, Ar-H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 20.85, 24.11, 24.46, 24.91, 25.92, 29.65, 63.44, 65.93, 70.67, 71.05, 96.28, 101.55, 108.72, 109.61, 120.26, 126.95, 128.26, 128.80, 129.54, 130.14, 131.66, 132.65, 164.11. Anal. Calcd for $C_{33}H_{28}N_2O_5$: C, 74.42; H, 5.29. Found: C, 74.38; H, 5.42.

11. Antimicrobial assay: All the newly synthesized compounds **5a–d**, **6**, and **9a–d** were screened *in vitro* for antibacterial activity against Gram-positive *Bacillus subtilis*, *Staphyllococcus aureus*, *Staphyllococcus epidermidis*,

Gram-negative *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* and for anti-fungal activity against *Saccharomyces cerevisiae*, *Aspergillus niger*, *Rhizopus oryzae* and *Candida albicans* by agar cup diffusion method.[12] The compounds showing some growth inhibition zone in this method were further tested by the broth dilution method[13] to determine their **MIC** values, which are summarized in Table 2. The synthesized compounds and reference drugs were dissolved in DMSO-H_2O (50%) at a concentration of 150 μg/mL. The concentrations were further made by twofold dilution with culture medium and bacterial solution at the first tube.

12. E. Margery Linday, Practical Introduction to Microbiology, E & F.N. Spon, UK (1962) 177.
13. NCCLS, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria, Which Grows Aerobically (fifth ed.), NCCLS, Villanova, PA (2000) Approved Standard M 7-A5.