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# Eco-Friendly Synthesis of Pyrimidine and Dihydropyrimidinone Derivatives under Solvent Free Condition and their Anti-microbial Activity

# Shaaban K Mohamed<sup>1,3</sup>\*, Ahmed M Soliman<sup>4</sup>, Mahmoud AA El-Remaily<sup>2,4</sup>, Hosam Abdel-Ghany<sup>4</sup>

<sup>1</sup>School of Chemistry and Environmental Science, Manchester Metropolitan University, Manchester M1 5GD, UK.

<sup>2</sup>Department of Organic Chemistry, Faculty of Science, Institute of Biotechnology, Granada University, Granada E-18071, Spain.

<sup>3</sup>Chemistry Department, Faculty of Science, Minia University, 61519 El-Minia, Egypt. <sup>4</sup>Department of Chemistry, Sohag University, 82524 Sohag, Egypt.

\*Correspondence: S.Mohamed@mmu.ac.uk

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#### Abstract

Reaction of 2-guanidinobenzimidazole and 2-guanidinebenzoxazole with several active methylene compounds revealed the formation of dihydropyrimidine and dihydropyrimidinone (DHPMs) derivatives *via* cyclo-condensation reaction. The products were obtained in a good yield, short time and a simple experimental procedure. The synthesized compounds were evaluated for their anti-bacterial activity against Gram-positive and Gram-negative bacteria. The compounds exhibited excellent zone of inhibition against tested bacteria. The investigation of antifungal screening data revealed that all the tested compounds showed moderate to good fungal inhibition. All compounds have been characterized based on IR, <sup>1</sup>H-NMR, <sup>13</sup>C-NMR and Mass spectrum data, in some cases the structure was established by X-Ray crystallography.

**Keywords:** Guanidinobenzimidazole; guanidinebenzoxazole; pyrimidine; dihydroprimidinone; active methylenes; anti-microbial activity.

#### 1. Introduction

Chemicals possessing pyrimidine and dihydro-pyrimidinone (DHPMs) entities showed a wide range of pharmacological properties including antiviral, antitumor, antibacterial, anti-inflammatory activities [1,2], calcium channel blocking [3], and neuropeptide Y (NPY) antagonistic activity [4]. DHPMs are used as therapeutics agents in the clinical treatment of cardiovascular diseases [5] such as hypertension [6], cardiac arrhythmias and orangina pectoris [7].

The synthetic methodology used to generate DHPMs has been well documented and has typically involved variations of the original Biginelli reaction [1, 11-13]. However, this reaction often requires harsh conditions and long reaction time and affords low yield, particularly when substituted aromatic and aliphatic aldehydes are employed. In recent years, several methods for the synthesis of DHPMs have been developed to improve and modify this reaction by means of microwave irradiation [14], ultrasound irradiation [15], ionic liquids [16], Lewis and protic acid promoters such as lanthanide triflate [17], H<sub>3</sub>BO<sub>3</sub> [18], VCl<sub>3</sub> [19], Sr(OTf)<sub>2</sub> [20], PPh<sub>3</sub> [21], Indium(III) halides [22], KAI(SO<sub>4</sub>)2·12H<sub>2</sub>O supported on silica [23], Silicasulfuric acid [24], Mn(OAc)3·2H<sub>2</sub>O [25], Y(NO<sub>3</sub>)3·6H<sub>2</sub>O [26], In(OTf)<sub>3</sub> [27], TaBr<sub>5</sub> [28], Ce(NO<sub>3</sub>)3·6H<sub>2</sub>O [29], silica chloride [30], HCOOH [31] and so on. However, in spite of their potential utility, many of these reported methodologies still have certain limitations such as expensive and air sensitive nature of catalysts, toxicity of solvents, restrictions for large scale applications, critical product isolation procedures, difficulty in recovery of high boiling solvents, excessive amounts of catalysts and generation of large amounts of toxic wastes in scaling up for industrial applications leading to environmental issues. Moreover, organic reactions under solvent free conditions have increasingly attracted chemists' interests, particularly from the view point of green chemistry [32-36]. Thus, the development of a simple and efficient method under solvent free and friendly catalyst condition for constructing these heterocyclic has been advocated.

# 2. Methods

All melting points are uncorrected and were recorded on Melt-Temp II melting point apparatus. IR spectra were measured as KBr pellets on a Shimadzu DR-8001 spectrometer. <sup>1</sup>H-NMR spectra were recorded on a Varian Gemini at 400 MHz using TMS as an internal reference and DMSO-*d6* as a solvent. Mass spectra were performed on a Shimadzu GCMS-QP 1000 mass spectrometer at 70 eV. The elemental analyses were carried out on a Perkin-Elmer 240C Microanalyzer. All compounds were checked for their purity on TLC plates. X-ray was measured on Bruker APEX2; cell refinement: Bruker SAINT; program(s) used to solve structure: SHELXS97; program(s) used to refine structure: SHELXL97; molecular graphics: XSEED.

### Modified procedure for preparation of 2-guanidinobenzimidazole 1<sub>a</sub> and 2-guanidinobenz-oxazole 1<sub>b</sub>

#### Method A

A mixture of o-phenylenediamine or o-aminophenol (1 mol.), dicyandiamide (1 mol.), concentrated hydrochloric acid (2 mols.) and water (15 c.c.) was heated under reflux for 3 hours. The reaction mixture allowed to cool down and treated with 10% NaOH to afford the crude products  $1_{a,b}$  respectively. The solid product has been recrystallized from CHCl<sub>3</sub> to afford pure needles in 95% yield.

#### Method B

*o*-phenylenediamine or *o*-aminophenol (50 mmol) were dissolved on heating in 50 mL of 10% sulfuric acid and dicyandiamide (75 mmol) was added. The reaction mixture was heated for 20 min and then 10 mL of 50% sodium hydroxide solution was added and heated for further 15 minutes. The reaction mixture was cooled and the obtained solid was collected by filtration and washed with water. The prepared compounds were sufficiently pure and used without further purification with 97% yield. Recrystallized samples have been prepared for analyses.

# General procedure for preparation of compounds (8<sub>a,b</sub>-13<sub>a,b</sub>)

A mixture of compound  $1_a$  or  $1_b$  (50 mmol) and 10 ml of acetylacetone, ethylcyanoacetate, ethyl acetoacetate, ethyl benzoylacetate or diethyl malonate in the presence of a few drops of catalytic glacial acetic acid was refluxed at 200°C. The solid products were observed after 15-20 min. The reaction proceeded with reflux for further 1 h. After cooling down, the solid crystalline products were filtered off, washed and recrystallized from ethanol.

# N-[(2Z)-4,6-dimethylpyrimidin-2(1H)-ylidene]-1H-benzimidazol-2-amine (8<sub>a</sub>)

Yield 83 %, mp 350 °C ; IR: cm<sup>-1</sup> 3257,3187 (2NH); <sup>1</sup>HNMR: δ 11.74 (s,1H, NH), 7.62-7.11 (br,5H, arom + NH), 6.85 (s,1H, CH) , 2.5 (s,6H, 2CH<sub>3</sub>); <sup>13</sup>CNMR: δ 19.82, 2015, 107.11, 115.01, 115.89, 122.77, 123.10, 139.13, 140.23, 147.50, 159.24, 161.55, 164.67; MS m/z (%): M<sup>+</sup> 239 (27.47), 132 (36.94), 107 (81.61), 67 (100); Anal.Calc. For C<sub>13</sub>H<sub>13</sub>N<sub>5</sub> (239.27):C(65.25%) H(5.48%) N(29.27%). Found: C(65.29%) H(5.62%) N(29.03%).

# N-[(2Z)-4,6-dimethylpyrimidin-2(1H)-ylidene]-1,3-benzoxazol-2-amine (8b)

Yield 77 %, mp 325 °C ; IR: cm<sup>-1</sup> 3278 (NH); <sup>1</sup>HNMR: δ, 7.47-6.44(br,5H, arom + NH), 6.23 (s,1H, CH) , 2.5 (s,6H, 2CH<sub>3</sub>); <sup>13</sup>CNMR: δ 19.78, 19.98, 107.02, 109.80, 116.45, 122.65, 126.12, 144.66, 147.12, 147.57, 150.45, 161.44, 169.10; MS m/z (%): M<sup>+</sup> 240 (39.22), 144 (56.15), 109 (100); Anal.Calc. For C<sub>13</sub>H<sub>12</sub>N<sub>4</sub>O (240.26), C(64.99%), H(5.03%) N(23.32%). Found: C(64.87%) H(4.98%) N(23.01%).

# (2Z)-6-amino-2-(1H-benzimidazol-2-ylimino)-2,3-dihydropyrimidin-4(1H)-one(9a)

Yield 91 %, mp >350 °C ; IR: cm<sup>-1</sup> 3411,3374,3266 (3NH,NH<sub>2</sub>),1657 (CO); <sup>1</sup>HNMR: δ 12.45 (s,1H, NH), 7.47-7.23 (br,5H, arom + NH), 6.67 (s,1H, CH) , 4.86 (s,1H, NH), 4.33 (s,2H, NH<sub>2</sub>); <sup>13</sup>CNMR: δ 85.66, 114.44, 114.87, 122.77, 123.55, 138.45, 139.40, 150.12, 158.47, 164.88, 165.07; MS m/z (%): M<sup>+</sup> 242 (2.20), 158 (31.08), 132 (44.75), 107 (27.56), 67 (100); Anal.Calc. For  $C_{11}H_{10}N_6O$  (242.24): C(54.54%) H(4.16%) N(34.69%). Found: C(54.61%) H(4.01%) N(34.71%).

#### (2Z)-6-amino-2-(1,3-benzoxazol-2-ylimino)-2,3-dihydropyrimidin-4(1H)-one (9<sub>b</sub>)

Yield 80 %, mp 210  $^{\circ}$ C ; IR: cm<sup>-1</sup> 3389,3280,3175 (2NH,NH<sub>2</sub>),1670 (CO); <sup>1</sup>HNMR: δ 9.87 (s,1H, NH), 9.52 (s,1H, NH), 6.96-6.74(br,4H, arom), 5.87 (s,1H, CH) , 4.02 (s,2H, NH<sub>2</sub>); <sup>13</sup>CNMR: δ 85.14, 109.15, 115.77, 122.44, 126.01,

135.11, 144.04, 149.89, 158.45, 164.22, 171.44; MS m/z (%):M<sup>+</sup> 243 (11.03), 176 (75.16), 136 (100), 109 (44.12); Anal.Calc. For C<sub>11</sub>H<sub>9</sub>N<sub>5</sub>O<sub>2</sub> (243.22): C(60.91%) H(4.72%) N(21.86%). Found: C(60.86%) H(4.61%) N(21.05%).

# Synthesis of (2E)-2-(1H-benzimidazol-2-ylimino)-1,2-dihydrop-yrimidine-4,6-diamine (10<sub>a</sub>)

Yield 82 %, mp 305°C ; IR: cm<sup>-1</sup> 3389,3274,3180 (2NH, 2NH<sub>2</sub>), <sup>1</sup>HNMR: δ, 11.95 (s,1H, NH), 7.91-7.07 (br,5H, arom+NH), 5.74 (s,4H, 2NH<sub>2</sub>), 5.51 (s,1H, CH); <sup>13</sup>CNMR: δ 95.12, 115.12, 115.78, 122.73, 123.44, 138.98, 139.69, 159.14, 161.88, 163.47, 166.66; Anal.Calc. For  $C_{11}H_{11}N_7$  (241.25), C(54.76%), H(4.60%) N(40.64%). Found: C(54.85%), H(4.72%) N(40.52%).

# Synthesis of (2E)-2-(1,3-benzoxazol-2-ylimino)-1,2-dihydropyr-imidine-4,6-diamine (10<sub>b</sub>)

Yield 78 %, mp 245<sup>o</sup>C ; IR: cm<sup>-1</sup> 3388,3274,3180 (NH,2NH<sub>2</sub>), <sup>1</sup>HNMR:  $\delta$ , 11.85 (s,1H, NH), 7.92-7.11 (br,4H, arom), 5.61 (s,4H, 2NH<sub>2</sub>), 5.43 (s,1H, CH); <sup>13</sup>CNMR:  $\delta$  94.87, 109.22, 116.68, 122.43, 126.10, 144.77, 144.95, 150.33, 163.04, 166.55, 169.74; Anal.Calc. For C<sub>11</sub>H<sub>10</sub>N<sub>6</sub>O (242.24), C(54.54%), H(4.16%) N(34.69%). Found: C(54.62%), H(4.23%) N(34.51%).

#### (2Z)-2-(1H-benzimidazol-2-ylimino)-6-methyl-2,3-dihydropyrimidin-4(1H)-one(11<sub>a</sub>)

Yield 88 %, mp >330 °C ; IR: cm<sup>-1</sup> 3365,3209 (2NH), 1651 (CO); <sup>1</sup>HNMR: δ 12.23 (s,1H, NH), 7.40-7.07 (br,5H, arom + NH), 5.54 (s,1H, CH) , 2.49 (s,3H, CH<sub>3</sub>); MS m/z (%): M<sup>+</sup> 241 (17.04), 158 (49.86), 132 (15.10), 107 (31.02), 67 (100); <sup>13</sup>CNMR: δ 19.01, 100.44, 114.07, 114.79, 122.77, 123.33, 138.23, 139.09, 150.66, 154.45, 164.89, 165.72; Anal.Calc. For C<sub>12</sub>H<sub>11</sub>N<sub>5</sub>O (241.25): C(59.74%) H(4.60%) N(29.03%). Found: C(59.93%) H(4.44%) N(28.87%).

#### (2Z)-2-(1,3-benzoxazol-2-ylimino)-6-methyl-2,3-dihydropyrimidin-4(1H)-one (11<sub>b</sub>)

Yield 80 %, mp 315 °C ; IR: cm<sup>-1</sup> 3323,3191(2NH),1661 (CO); <sup>1</sup>HNMR:  $\delta$  12.30 (s,1H, NH), 7.56-7.16 (br,5H, arom + NH), 5.68 (s,1H, CH) , 2.51 (s,3H, CH<sub>3</sub>); <sup>13</sup>CNMR:  $\delta$  18.97, 100.14, 109.48, 115.11, 122.59, 126.48, 137.14, 144.39, 149.88, 150.23, 165.32, 171.40; MS m/z (%): M<sup>+</sup> 242 (100), 160 (43.57), 134 (28.19), 109 (64.84); Anal.Calc. For C<sub>12</sub>H<sub>10</sub>N<sub>4</sub>O<sub>2</sub> (242.23): C(59.50%) H(4.16%) N(23.13%). Found: C(59.61%) H(4.03%) N(23.01%).

### (2Z)-2-(1H-benzimidazol-2-ylimino)-6-phenyl-2,3-dihydropyrimidin-4(1H)-one (12<sub>a</sub>)

Yield 68 %, mp >360 °C ; IR: cm<sup>-1</sup> 3214,3185 (3NH), 1658 (CO); <sup>1</sup>HNMR:  $\delta$  12.09 (s,1H, NH), 8.01-7.18 (br,11H, 2arom + 2NH), 6.29 (s,1H, CH); <sup>13</sup>CNMR:  $\delta$  101.70, 114.14, 114.79, 122.33, 123.71, 127.02, 127.63, 130.22, 136.10, 138.02, 139.46, 151.46, 154.22, 164.98, 165.37; MS m/z (%): M<sup>+</sup> 303 (78.33), 158 (100), 133 (22.74), 103 (27.82), 77 (37.26); Anal.Calc. For C<sub>17</sub>H<sub>13</sub>N<sub>5</sub>O (303.32): C(67.32%) H(4.32%) N(23.09%). Found: C(67.44%) H(4.07%) N(23.11%).

# (2E)-2-(1,3-benzoxazol-2-ylimino)-6-phenyl-2,3-dihydropyrimidin-4(1H)-one (12<sub>b</sub>)

Yield 85 %, mp 220  $^{\circ}$ C ; IR: cm<sup>-1</sup> 3381,3207(2NH),1678 (CO); <sup>1</sup>HNMR: δ 12.19 (s,1H, NH), 7.98-7.24 (br,10H, 2arom + NH), 6.27 (s,1H, CH); <sup>13</sup>CNMR: δ 101.10, 109.19, 115.77, 122.49, 126.33, 127.18, 127.98, 130.23, 135.12, 137.22, 144.06, 149.78, 151.04, 164.95, 171.76; MS m/z (%): M<sup>+</sup> 304 (76.18), 159 (100), 134 (45.15), 109 (37.11); Anal.Calc. For C<sub>17</sub>H<sub>12</sub>N<sub>4</sub>O<sub>2</sub> (304.30): C(67.10%) H(3.97%) N(18.41%). Found: C(67.28%) H(3.44%) N(18.29%).

# (2Z)-2-(1H-benzimidazol-2-ylimino)-6-hydroxy-2,3-dihydropyrimidin-4(1H)-one (13<sub>a</sub>)

Yield 68 %, mp 330 °C ; IR: cm<sup>-1</sup> 3438 (OH), 3245,3218 (3NH), 1693 (CO); <sup>1</sup>HNMR: δ 12.00 (s,1H, NH), 10.09 (s,1H, OH), 9.47 (s,1H, NH), 7.98-7.05 (br,5H, arom + NH), 6.34 (s,1H, CH); <sup>13</sup>CNMR: δ 88.10, 114.07, 114.56, 122.77, 123.23, 138.44, 139.70, 154.17, 164.01, 164.88, 165.67; MS m/z (%): M<sup>+</sup> 243 (10.35), 174 (72.71), 132 (78.11), 107 (39.66), 77 (100); Anal.Calc. For  $C_{11}H_9N_5O_2$  (243.22): C(54.32%) H(3.73%) N(28.79%). Found: C(54.77%) H(3.56%) N(28.48%).

#### (2Z)-2-(1,3-benzoxazol-2-ylimino)-6-hydroxy-2,3-dihydropyrimidin-4(1H)-one (13b)

Yield 85 %, mp 220 °C ; IR: cm<sup>-1</sup> 3428 (OH), 3295,3161(2NH),1656 (CO); <sup>1</sup>HNMR: δ 12.07 (s,1H, NH), 9.98 (s,1H, OH), 7.57-7.27 (br,5H, arom + NH), 5.85 (s,1H, CH); MS m/z (%): M<sup>+</sup> 244 (16. 81), 176 (100), 134 (33.11), 109 (73.13); <sup>13</sup>CNMR: δ 88.89, 109.47, 115.23, 122.77, 126.47, 137.12, 144.05, 149.48, 164.10, 164.80, 171.23; Anal.Calc. For C<sub>11</sub>H<sub>8</sub>N<sub>4</sub>O<sub>3</sub> (244.21): C(54.10%) H(3.30%) N(22.94%). Found: C(54.37%) H(3.09%) N(22.79%).

# 3. Results and Discussion

Reaction of *o*-phenylenediamine or *o*-aminophenol with cyanoguanidine in conc. HCl gave the corresponding 2-guanidinobenzimidazole  $\mathbf{1}_{a}$  [37] and 2-guanidinobenzoxazole  $\mathbf{1}_{b}$  [38] respectively (Figures 1, 2). Compound  $\mathbf{1}_{a}$  was also prepared *via* another rout by using conc. H<sub>2</sub>SO<sub>4</sub> [39]. IR spectra of compounds  $\mathbf{1}_{b}$  showed new absorption band corresponding to NH, NH<sub>2</sub> at 3449 – 3144Cm<sup>-1</sup>, <sup>1</sup>H-NMR spectra of compounds  $\mathbf{1}_{b}$  showed a signal at 8.90 ppm corresponding to NH group, 6.34-7.53 ppm for both aromatic and NH group. NH<sub>2</sub> group has been observed at 4.47 ppm (Scheme 1).





When compounds  $\mathbf{1}_{a,b}$  reacted with acetylacetone in presence of few drops of glacial acetic acid as a benign catalyst afforded pyrimidine derivatives  $\mathbf{8}_{a,b}$ . IR spectra of compounds  $\mathbf{8}_{a,b}$  showed disappearance of absorption bands corresponding to NH and NH<sub>2</sub> groups. Their <sup>1</sup>HNMR spectra showed new signals corresponding

to NH groups at 6.85, 6.23 ppm and 2CH<sub>3</sub> at 2.5 ppm respectively. Mass spectra of compound  $\mathbf{8}_{a,b}$  gave molecular ion peaks at m/z 239 and 240, respectively.



#### Figure 3: X-ray monocrystal structure and crystal latix of 8a.

#### Crystal data and structure refinement of compound (8<sub>a</sub>) [40] (Figure 3)

Empirical formula  $C_{13}H_{13}N_5$ ; Formula weight 239.27; Independent reflections 5975 [R(int) = 0.0184]; Temperature 296(2) K; Completeness to theta = 28.34° and 99.2 %; Wavelength 0.71073 Å; Max. and min. transmission 0.9833 and 0.9750; Space group P-1; Density (calculated) 1.317 Mg/m<sup>3</sup>; Absorption coefficient 0.085 mm<sup>-1</sup>; Crystal size: 0.30 x 0.20 x 0.20 mm<sup>3</sup>.

There are two independent molecules in the asymmetric unit of the title compound,  $C_{13}H_{13}N_5$ . In each molecule, an amino N atom is connected to a benzimidazole fused-ring system and a pyrimidine ring [these are aligned at 1.3 (1)<sup>o</sup> in one independent molecule and at 5.4 (1)<sup>o</sup> in the other]. The amino N atom of the fused ring forms an intramolecular N–H....O hydrogen bond to a pyrimidine N atom in each molecule. The amino N atom connecting the two ring systems interacts with the other N atom of the pyrimidine ring of an adjacent molecule, generating centrosymmetric hydrogen-bonded dimers.

#### Selected bond length [Å] and angles [°] in (8<sub>a</sub>)

O(1)-C(5) 1.230(2), O(2)-C(9) 1.243(2), C(8)-C(9) 1.448(2), C(5)-C(6) 1.464(2), C(8)-C(13) 1.361(2), C(1)-C(6) 1.355(2), N(1)-C(13) 1.397(2),N(1)-C(1) 1.402(2), O(4)-C(25) 1.365(3). O(2)-C(9)-C(8) 121.57(16), O(1)-C(5)-C(6) 120.90(16), C(13)-C(8)-C(9) 121.00(15), C(1)-C(6)-C(5) 121.01(15), C(8)-C(13)-N(1) 120.61(14), C(6)-C(1)-N(1) 120.32(15), C(13)-N(1)-C(1) 119.00(13), C(5)-C(6)-C(7) 117.03(14).

It has been reported that the reaction of biguanides with ethyl cyanoacetate gave triazineacetonitrile [41]. Reaction of  $\mathbf{1}_{a}$  or  $\mathbf{1}_{b}$  with ethyl cyanoacetate gave 2,3-dihydropyrimidin-4(1H)-ones  $\mathbf{9}_{a}$  or  $\mathbf{9}_{b}$  respectively (Scheme 2). Their reaction mechanism was proceeding *via* condensation reaction between amino group and ester group with elimination of ethanol molecule followed by nucleophilic addition of the amino group on cyano group. IR spectra of compounds  $\mathbf{9}_{a,b}$  showed new absorption bands corresponding to NH<sub>2</sub> groups at 3280– 3411 Cm<sup>-1</sup> and for C=O groups at 1657 and 1670 Cm<sup>-1</sup> respectively. MS of compounds  $\mathbf{9}_{a,b}$  showed molecular ion peaks at m/z 242 and 243, respectively.

Similarly, a nucleophilic addition of the two amino of guanidyl group in compounds  $\mathbf{1}_{a,b}$  to the two cyano groups in malononitrile afforded the corresponding hydropyrimidines  $\mathbf{10}_{a,b}$  respectively. IR spectra for the later showed new absorption bands corresponding to NH<sub>2</sub> groups at 3389– 3274 Cm<sup>-1</sup> and signals at 5.61- 5.74 ppm in <sup>1</sup>H-NMR spectra for 2-NH<sub>2</sub> groups.

6-Methyl-, 6-phenyl and 6-hydroxy-2,3-dihydropyrimidin-4(1H)-ones  $\mathbf{11}_{a,b}-\mathbf{13}_{a,b}$  were synthesized *via* reaction of  $\mathbf{1}_a$  or  $\mathbf{1}_b$  with ethyl acetoacetate, ethyl benzoylacetate or diethyl malonate in presence of the benign catalyst glacial acetic acid (Scheme 2). IR spectra of compounds  $\mathbf{11}_{a,b} - \mathbf{13}_{a,b}$  showed new absorption bands corresponding to C=O groups at 1693-1656 Cm<sup>-1</sup>. Their <sup>1</sup>HNMR spectra showed new signals corresponding to =CH groups at 6.34 - 5.54 ppm. Mass spectra of compounds  $\mathbf{11}_{a,b} - \mathbf{13}_{a,b}$  showed molecular ion peaks at m/z 241, 242, 303, 304, 243 and 244 respectively.

Scheme 2: Reaction illustration of active methylenes (2-7) with 2-guanidinobenzimidazole 1<sub>a</sub> and 2-guanidinebenzoxazole 1<sub>b</sub>.



#### 4. Anti-bacterial activity

All compounds were dissolved in DMSO. In order to ensure that the solvent had no effect on bacterial growth or enzymatic activity, negative control tests were performed using DMSO at the same concentrations.

The inhibitory effect of compounds  $1_{a,b}$ ,  $8_{a,b} - 13_{a,b}$  on the *in vitro* growth of broad spectrum of bacteria representing different types of Gram-positive and Gram-negative bacteria, namely *Bacillus cereus, Bacillus subtilis, Escherichia coli, Micrococcus luteus, Staphylococcus aureus, Pseudomonas aeruginosa* and *Micrococcus roseus* was evaluated using agar diffusion method (cup and plate method) [42]. DMSO was used as solvent control. All plates were incubated at  $37\pm0.5^{\circ}$ C for 24h. The zone of inhibition of compounds was measured using cm scale. The results in Table 1 revealed that most compounds at high concentration (50.000 ppm) showed generally a good inhibitory effect against all types of bacteria. **9**<sub>a</sub> showed the highest inhibition zone against both of gram positive and gram negative bacteria while compound **9**<sub>b</sub> showed the lowest one.

Types of Bacteria	Compound 1 <sub>a</sub>			Compound 1 <sub>b</sub>			Compound 8 <sub>a</sub>		
	Concentrations			Concentrations			Concentrations		
-	10000	30000	50000	10000	30000	50000	10000	30000	50000
	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm
D									
Bacillus cereus	0.8 cm	1.2 cm	1.5 cm	0.7 cm	1.1 cm	1.5 cm	0.4 cm	0.8 cm	1.0 cm
Bacillus subtilis	0.4 cm	0.6 cm	0.9 cm	0.1 cm	0.2 cm	0.4 cm	1 cm	1.2 cm	1.3 cm
Escherichia coli	0.6 cm	0.9 cm	1.4 cm	0.8 cm	1.2 cm	1.8 cm	0.5 cm	0.6 cm	1.0 cm
Micrococcus luteus	0.3 cm	0.6 cm	1.0 cm	0.2 cm	0.5 cm	0.7 cm	0.4 cm	0.7 cm	0.9 cm
Staphylococcus	010 0111	010 011	210 0111						0.0 0
aureus	0.2 cm	0.5 cm	0.8 cm		0.2 cm	0.6 cm	0.5 cm	0.9 cm	1.1 cm
Pseudomonas	0.1 cm	0.2 cm	0.0 cm	0.1 cm	0.4 cm	0.8 cm	0.6 cm	0.8 cm	0.0 cm
Micrococcus roseus	0.1 (11)	0.5 CIII	0.9 CIII	0.1 cm	0.4 cm	0.8 cm	0.0 011	0.8 011	0.9 011
	0.2 cm	0.4 cm	0.7 cm	0.5 cm	0.0 011	0.5 011	0.2	0.6 cm	0.9 cm
Types of Bacteria	Compound 8 <sub>b</sub>			Compound 9 <sub>a</sub>			Compound 9 <sub>b</sub>		
	Concentrations			Concentrations			Concentrations		
-	10000	30000	50000	10000	30000	50000	10000	30000	50000
	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm
Bacillus cereus									
	0.4 cm	0.6 cm	0.7 cm	0.4 cm	0.7 cm	1.0 cm	0.6 cm	0.7 cm	1.0 cm
Bacillus subtilis	0.5 cm	0.7 cm	1.0 cm	1.0 cm	1.1 cm	1.5 cm	1 cm	1.5 cm	1.9 cm
Escherichia coli									
	0.6	0.9	1.2 cm	0.8 cm	1.0 cm	1.5 cm	1.1 cm	1.5 cm	1.7 cm
Micrococcus luteus		0.5 cm	0.7 cm	0.5 cm	0.9 cm	1.2 cm		0.6 cm	0.9 cm
Staphylococcus									
aureus			0.9 cm	0.4 cm	0.7 cm	0.9 cm	0.6 cm	0.7 cm	0.7 cm
Pseudomonas									
acragiiosa	0.4	0.8 cm	1.0 cm	0.7 cm	1.0 cm	1.4 cm	0.6 cm	1.0 cm	1.2 cm

Table 1: Results of anti-bacterial evaluation of compounds 1<sub>a,b</sub>,8<sub>a,b</sub>-13<sub>a,b</sub>.

Types of Bacteria	Compound 11 <sub>a</sub>			Compound 11 <sub>b</sub>			Compound 12 <sub>a</sub>		
	Concentrations			Concentrations			Concentrations		
	10000	30000	50000	10000	30000	50000	10000	30000	50000
	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm
Bacillus cereus									
	0.4 cm	0.6 cm	0.9 cm	0.4 cm	0.8 cm	0.9 cm	0.7 cm	1.0 cm	1.4 cm
Bacillus subtilis	0.6 cm	0.9 cm	1.1 cm	0.5 cm	0.8cm	1.3 cm	0.5 cm	0.9 cm	1.3 cm
Escherichia coli	0.5 cm	0.8 cm	1.1 cm	0.3 cm	0.7 cm	1.0 cm	0.5 cm	0.7 cm	1.0 cm
Micrococcus luteus		0.4 cm	1.0 cm			0.6 cm	0.2 cm	0.5 cm	0.7 cm
Staphylococcus		0.5			0.5				
aureus	0.3 cm	0.5 cm	0.8 cm		0.5 cm	0.8 cm	0.4 cm	0.7 cm	0.9 cm
Pseudomonas									
aeruginosa	0.6 cm	0.7 cm	0.9 cm	0.4 cm	0.8 cm	0.8 cm	0.5 cm	0.6 cm	0.9 cm
Micrococcus roseus	0.8 cm	0.8 cm	13 cm	0.5 cm	0.9 cm	1.1 cm	0.5 cm	0.9 cm	14 cm
Types of Bacteria	C	ompound 12	2 <sub>b</sub>	C	ompound 1	<b>B</b> a	C	ompound 1	3 <sub>b</sub>
	Concentrations			Concentrations			Concentrations		
	concentrations			concentrations					
	10000	30000	50000	10000	30000	50000	10000	30000	50000
	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm
Bacillus cereus	0.3 cm	0.6.cm	0.7 cm	0.4 cm	0.6.cm	1.0 cm	0.5 cm	0.7 cm	0 9 cm
Bacillus subtilis				0. T citi	0.0 0.11	1.0 0.11			0.5 cm
Facharichia cali		0.6 cm	1.0 cm	0.4 cm	0.7 cm	1.1 cm	0.5 cm	0.7 cm	1.0 cm
Escherichia con	0.5 cm	0.8 cm	1.2 cm	1.0 cm	1.4 cm	1.8 cm	0.7	0.9	1.3 cm
Micrococcus luteus	0.2 cm	0.3 cm	0.8 cm	0.5 cm	0.8 cm	1.4 cm	0.3 cm	0.5 cm	0.7 cm
Staphylococcus	0.2 cm	0.5 cm	0.8 cm	0.5 cm	0.8 cm	1.4 Cm	0.5 cm	0.5 cm	0.7 cm
aureus		0.2 cm	0.6 cm	0.8 cm	0.9 cm	1.5 cm		0.2 cm	0.6 cm
Pseudomonas	0.7 cm	0.8 cm	0.0 am	0.4 am	0.5 am	0.8 am	0.5	0.7.000	1.1.000
aeruginosa	0.7 cm	0.8 CM	0.9 CM	0.4 CM	0.5 CM	0.8 CM	0.5	0.7 cm	1.1 CM
Micrococcus roseus	0.4 cm	0.5 cm	1.0 cm	0.4 cm	0.5 cm	0.9 cm	0.6 cm	0.9 cm	1.1 cm

# 5. Antifungal studies

Antifungal activity was also studied by disk diffusion method. For assaying antifungal activity *Penicillium purpurogenium*, *Aspergillus flavus* and *Trichotheium rosium* were inoculated in Sabouraud Dextrose broth medium (Hi-Media Mumbai) and incubated for 48–72 h at 35°C, and subsequently, a suspension of about 1.6 X  $10^4$ -6 X  $10^4$  c.f.u./mL was introduced on to the surface of sterile agar plates, and a sterile glass spreader was used for even distribution of the inoculum. The discs measuring 6 mm in diameter were prepared from Whatmann No. 1 filter paper and sterilized by dry heat at  $140^{\circ}$ C for 1 h.

Fluconazole (30 mg/mL) was used as positive control while the disk poured in DMSO was used as negative control. The plates were inverted and incubated for 48–72 h at 35°C. The susceptibility was assessed on the basis of diameter of zone of inhibition against albicans and non-albicans strains of fungi. The investigation of antifungal screening data revealed that all the tested compounds showed moderate to good fungal inhibition.  $\mathbf{9}_a$  and  $\mathbf{13}_b$  are exhibited the highest fungal inhibitory effect while  $\mathbf{11}_b$  showed the lowest. Inhibition zones were measured and compared with the controls. The fungal zones of inhibition values are given in Table 2.

Types of Fungi	Compound 1a			Compound 1b			Compound 8a			
	C	Concentrations Concentrations			ns	Concentrations				
	10000	30000	50000	10000	30000	50000	10000	30000	50000	
	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	
Penicillium	-	-	+	-	-	+	-		+	
purpurogenium										
Aspergillus flavus	-	+	+	-	-	-	-	+	+	
Trichotheium rosium	-	-	+	-	+	++	-	-	+	
Types of Fungi	C	Compound 8b		Compound 9a			Compound 9b			
	C	oncentratio	ns	C	oncentratio	ns	Concentrations			
	10000	30000	50000	10000	30000	50000	10000	30000	50000	
	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	
Penicillium	-	-	+	-	+	++	+	++	++	
purpurogenium										
Aspergillus flavus	-	-	-	+	+	++	-	-	+	
Trichotheium rosium	-	-	+	-	+	+++	-	+	+	
Types of Fungi	Compound 10a			Compound 10b			Compound 11a			
	Concentrations			Concentrations			Concentrations			
	10000	30000	50000	10000	30000	50000	10000	30000	50000	
	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	
Penicillium	-	-	-	-	+	+	-	-	+	
purpurogenium										
Aspergillus flavus	-	-	+	+	+	+	-	-	-	
Trichotheium rosium	-	+	+	+	++	+	-	+	++	
Types of Fungi	Compound 11b			Compound 12a			Compound 12b			
	Concentrations			Concentrations			Concentrations			
	10000	30000	10000	10000	10000	50000	10000	10000	50000	
	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	
Penicillium	-	-	-	-	-	+	-	-	+	
purpurogenium										
Aspergillus flavus	-	-	-	-	+	+	-	-	+	
Trichotheium rosium	-	+	+	-	-	+	-	+	+	
Types of Fungi	Compound 13a			Compound 13b						
	Concentrations			Concentrations						
	10000	30000	10000	10000	10000	50000				
	ppm	ppm	ppm	ppm	ppm	ppm				
Penicillium	+	+	++	-	+	+				
purpurogenium										
Aspergillus flavus	-	++	++	-	-	+				
Trichotheium rosium	+	+	+	-	-	+				

#### Table 2: Results of antifungal activity of compounds $1_{a,b}$ and $8_{a,b} - 13_{a,b}$ .

#### **Competing Interests**

The authors have no competing interests with anyone.

#### **Authors' Contributions**

All authors are in equal contribution in different forms.

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