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

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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, ^{1}H-NMR, ^{13}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_3BO_3 [18], VCl_3 [19], $Sr(OTf)_2$ [20], PPh_3 [21], Indium(III) halides [22], $KAl(SO_4)2{\cdot}12H_2O$ supported on silica [23], Silicasulfuric acid [24], $Mn(OAc)3{\cdot}2H_2O$ [25], $Y(NO_3)3{\cdot}6H_2O$ [26], $In(OTf)_3$ [27], $TaBr_5$ [28], $Ce(NO_3)3{\cdot}6H_2O$ [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. ^{1}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_a and 2-guanidinobenz-oxazole 1_b

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 $CHCl_3$ 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 ($8_{a,b}$-$13_{a,b}$)

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_a)

Yield 83 %, mp 350 ºC ; IR: cm^{-1} 3257,3187 (2NH); ^{1}HNMR: δ 11.74 (s,1H, NH), 7.62-7.11 (br,5H, arom + NH), 6.85 (s,1H, CH) , 2.5 (s,6H, $2CH_3$); ^{13}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$^+$ 239 (27.47), 132 (36.94), 107 (81.61), 67 (100); Anal.Calc. For $C_{13}H_{13}N_5$ (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 (8_b)

Yield 77 %, mp 325 ºC ; IR: cm^{-1} 3278 (NH); ^{1}HNMR: δ, 7.47-6.44(br,5H, arom + NH), 6.23 (s,1H, CH) , 2.5 (s,6H, $2CH_3$); ^{13}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$^+$ 240 (39.22), 144 (56.15), 109 (100); Anal.Calc. For $C_{13}H_{12}N_4O$ (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(9_a)

Yield 91 %, mp >350 ºC ; IR: cm^{-1} 3411,3374,3266 (3NH,NH_2),1657 (CO); ^{1}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_2); ^{13}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$^+$ 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_b)

Yield 80 %, mp 210 ºC ; IR: cm^{-1} 3389,3280,3175 (2NH,NH_2),1670 (CO); ^{1}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_2); ^{13}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^{+} 243 (11.03), 176 (75.16), 136 (100), 109 (44.12); Anal.Calc. For $C_{11}H_{9}N_{5}O_{2}$ (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_{a})

Yield 82 %, mp 305ºC ; IR: cm^{-1} 3389,3274,3180 (2NH, $2NH_{2}$), ^{1}HNMR: δ, 11.95 (s,1H, NH), 7.91-7.07 (br,5H, arom+NH), 5.74 (s,4H, $2NH_{2}$), 5.51 (s,1H, CH); ^{13}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_{b})

Yield 78 %, mp 245ºC ; IR: cm^{-1} 3388,3274,3180 (NH,$2NH_{2}$), ^{1}HNMR: δ, 11.85 (s,1H, NH), 7.92-7.11 (br,4H, arom), 5.61 (s,4H, $2NH_{2}$), 5.43 (s,1H, CH); ^{13}CNMR: δ 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 $C_{11}H_{10}N_{6}O$ (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_{a})

Yield 88 %, mp >330 ºC ; IR: cm^{-1} 3365,3209 (2NH), 1651 (CO); ^{1}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_{3}); MS m/z (%): M^{+} 241 (17.04), 158 (49.86), 132 (15.10), 107 (31.02), 67 (100); ^{13}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 $C_{12}H_{11}N_{5}O$ (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_{b})

Yield 80 %, mp 315 ºC ; IR: cm^{-1} 3323,3191(2NH),1661 (CO); ^{1}HNMR: δ 12.30 (s,1H, NH), 7.56-7.16 (br,5H, arom + NH), 5.68 (s,1H, CH) , 2.51 (s,3H, CH_{3}); ^{13}CNMR: δ 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^{+} 242 (100), 160 (43.57), 134 (28.19), 109 (64.84); Anal.Calc. For $C_{12}H_{10}N_{4}O_{2}$ (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_{a})

Yield 68 %, mp >360 ºC ; IR: cm^{-1} 3214,3185 (3NH), 1658 (CO); ^{1}HNMR: δ 12.09 (s,1H, NH), 8.01-7.18 (br,11H, 2arom + 2NH), 6.29 (s,1H, CH); ^{13}CNMR: δ 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^{+} 303 (78.33), 158 (100), 133 (22.74), 103 (27.82), 77 (37.26); Anal.Calc. For $C_{17}H_{13}N_{5}O$ (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_{b})

Yield 85 %, mp 220 ºC ; IR: cm^{-1} 3381,3207(2NH),1678 (CO); ^{1}HNMR: δ 12.19 (s,1H, NH), 7.98-7.24 (br,10H, 2arom + NH), 6.27 (s,1H, CH); ^{13}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^{+} 304 (76.18), 159 (100), 134 (45.15), 109 (37.11); Anal.Calc. For $C_{17}H_{12}N_{4}O_{2}$ (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_{a})

Yield 68 %, mp 330 ºC ; IR: cm^{-1} 3438 (OH), 3245,3218 (3NH), 1693 (CO); ^{1}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); ^{13}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^{+} 243 (10.35), 174 (72.71), 132 (78.11), 107 (39.66), 77 (100); Anal.Calc. For $C_{11}H_{9}N_{5}O_{2}$ (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 (13_{b})

Yield 85 %, mp 220 ºC ; IR: cm^{-1} 3428 (OH), 3295,3161(2NH),1656 (CO); ^{1}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^{+} 244 (16. 81), 176 (100), 134 (33.11), 109 (73.13); ^{13}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 $C_{11}H_{8}N_{4}O_{3}$ (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 **1_a** [37] and 2-guanidinobenzoxazole **1_b** [38] respectively (Figures 1, 2). Compound **1_a** was also prepared *via* another rout by using conc. H_2SO_4 [39]. IR spectra of compounds **1_b** showed new absorption band corresponding to NH, NH_2 at 3449 – 3144Cm^{-1}, ^{1}H-NMR spectra of compounds **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_2 group has been observed at 4.47 ppm (Scheme 1).

Scheme 1: Synthesis illustration of 2-guanidinobenzimidazole and 2-guanidinebenzoxazole.

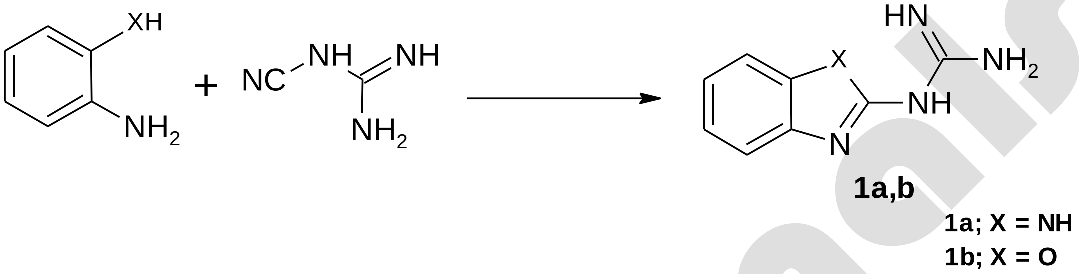


Figure 1: X-ray crystal structure of the acetate salt of 1_a.

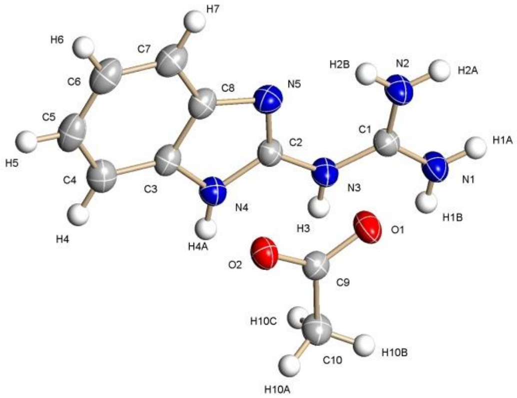
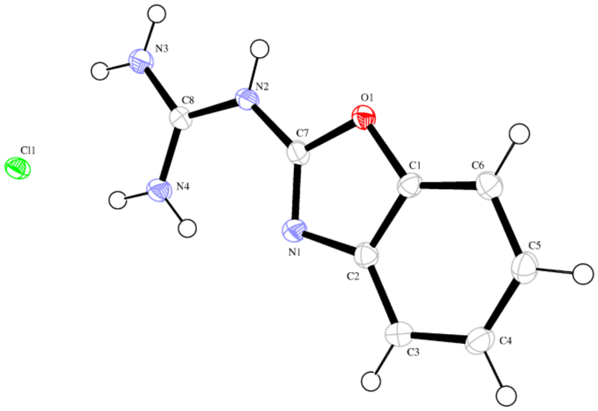
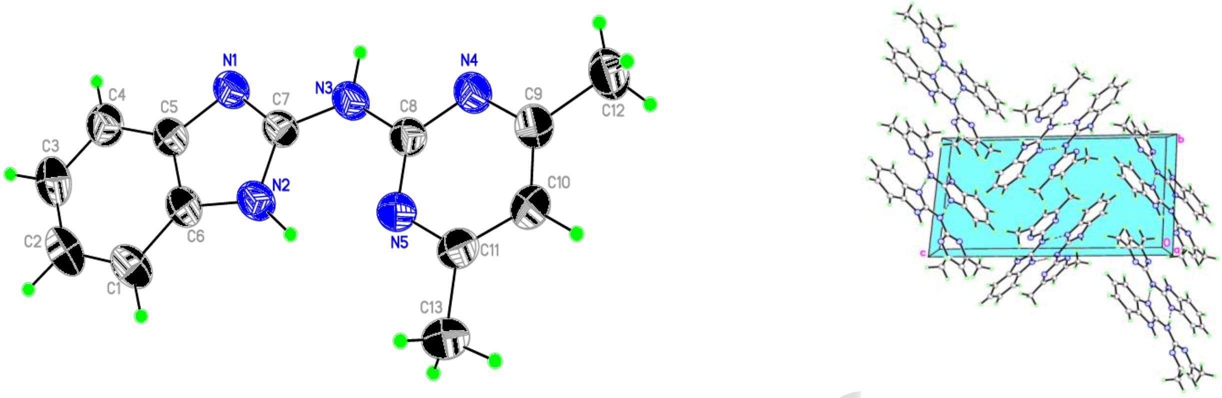


Figure 2: X-ray crystal structure of the chlorine salt of 1_b.



When compounds **$1_{a,b}$** reacted with acetylacetone in presence of few drops of glacial acetic acid as a benign catalyst afforded pyrimidine derivatives **$8_{a,b}$**. IR spectra of compounds **$8_{a,b}$** showed disappearance of absorption bands corresponding to NH and NH_2 groups. Their ^{1}HNMR spectra showed new signals corresponding to NH groups at 6.85, 6.23 ppm and $2CH_3$ 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_a) [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^3; Absorption coefficient 0.085 mm^{-1}; Crystal size: 0.30 x 0.20 x 0.20 mm^3.

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)^o in one independent molecule and at 5.4 (1)º 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_a)

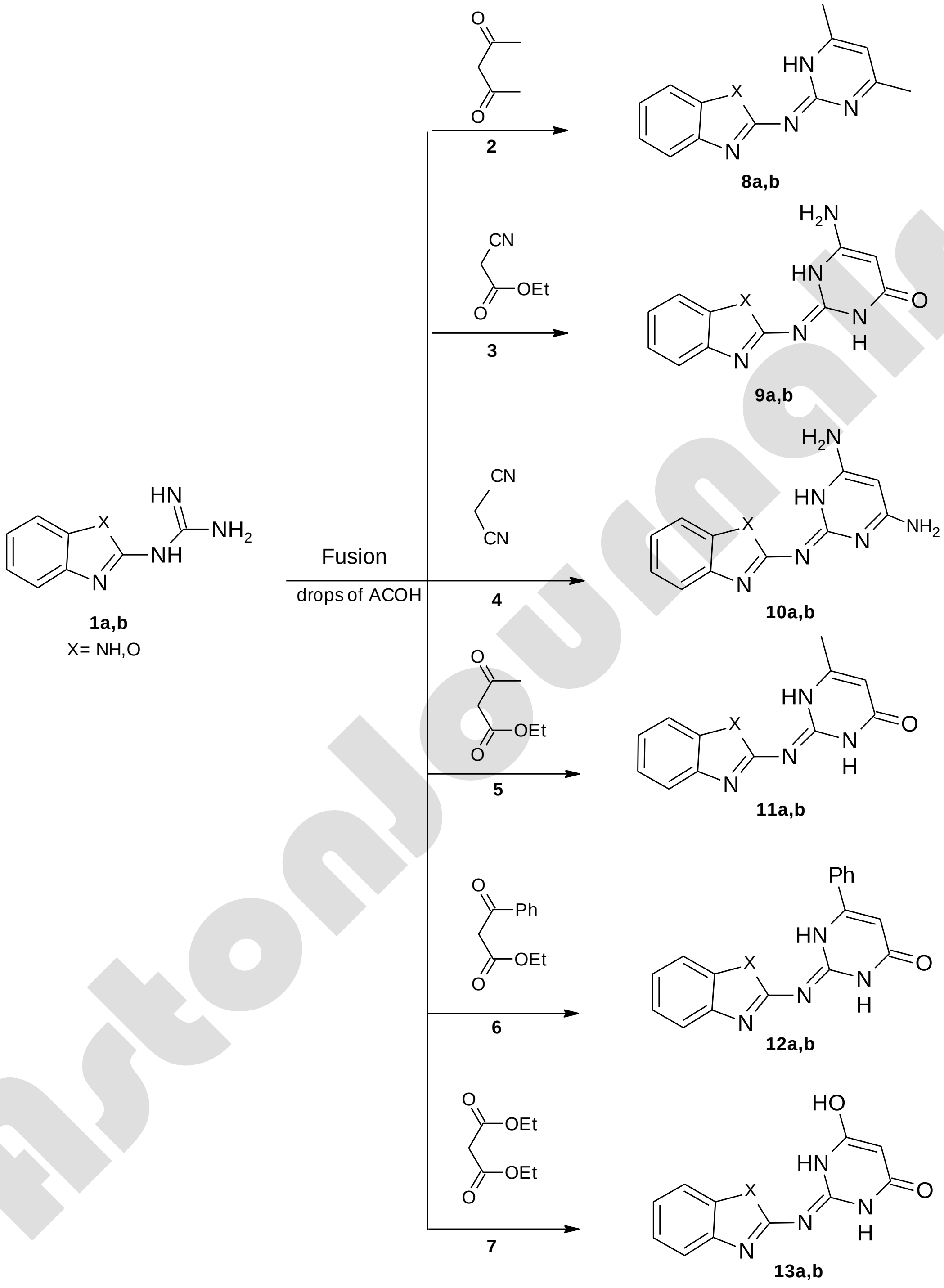
O(1)-C(5) 1.230(2), O(2)-C(9) 1.243(2), C(8)-C(9) 1.448(2), C(5)-C(6) 1.464(2), C(8)-C(13) 1.361(2), C(1)-C(6) 1.355(2), N(1)-C(13) 1.397(2),N(1)-C(1) 1.402(2), O(4)-C(25) 1.365(3). O(2)-C(9)-C(8) 121.57(16), O(1)-C(5)-C(6) 120.90(16), C(13)-C(8)-C(9) 121.00(15), C(1)-C(6)-C(5) 121.01(15), C(8)-C(13)-N(1) 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_2 groups at 3280– 3411 Cm^{-1} and for C=O groups at 1657 and 1670 Cm^{-1} 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_2 groups at 3389– 3274 $Cm^{-1}$and signals at 5.61- 5.74 ppm in ^{1}H-NMR spectra for 2-NH_2 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^{-1}. Their ^{1}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_a and 2-guanidinebenzoxazole 1_b.



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±0.5^{o}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_a** showed the highest inhibition zone against both of gram positive and gram negative bacteria while compound **9_b** showed the lowest one.

Table 1: Results of anti-bacterial evaluation of compounds $1_{a,b}$,$8_{a,b}$-$13_{a,b}$.

Types of Bacteria	**Compound 1_a**			**Compound 1_b**			**Compound 8_a**		
	Concentrations			**Concentrations**			**Concentrations**		
	10000 ppm	30000 ppm	50000 ppm	10000 ppm	30000 ppm	50000 ppm	10000 ppm	30000 ppm	50000 ppm
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 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 aeruginosa	0.1 cm	0.3 cm	0.9 cm	0.1 cm	0.4 cm	0.8 cm	0.6 cm	0.8 cm	0.9 cm
Micrococcus roseus	0.2 cm	0.4 cm	0.7 cm	0.3 cm	0.5 cm	0.9 cm	0.2	0.6 cm	0.9 cm
Types of Bacteria	**Compound 8_b**			**Compound 9_a**			**Compound 9_b**		
	Concentrations			**Concentrations**			**Concentrations**		
	10000 ppm	30000 ppm	50000 ppm	10000 ppm	30000 ppm	50000 ppm	10000 ppm	30000 ppm	50000 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 aeruginosa	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
Micrococcus roseus	0.4 cm	0.7 cm	1.1 cm	0.6 cm	0.9 cm	1.4 cm	0.3 cm	0.5 cm	1.0 cm

Types of Bacteria	**Compound 11_a**			**Compound 11_b**			**Compound 12_a**		
	Concentrations			**Concentrations**			**Concentrations**		
	10000 ppm	30000 ppm	50000 ppm	10000 ppm	30000 ppm	50000 ppm	10000 ppm	30000 ppm	50000 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 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	1.3 cm	0.5 cm	0.9 cm	1.1 cm	0.5 cm	0.9 cm	1.4 cm
Types of Bacteria	**Compound 12_b**			**Compound 13_a**			**Compound 13_b**		
	Concentrations			**Concentrations**			**Concentrations**		
	10000 ppm	30000 ppm	50000 ppm	10000 ppm	30000 ppm	50000 ppm	10000 ppm	30000 ppm	50000 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	---	0.6 cm	1.0 cm	0.4 cm	0.7 cm	1.1 cm	0.5 cm	0.7 cm	1.0 cm
Escherichia coli	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 aureus	---	0.2 cm	0.6 cm	0.8 cm	0.9 cm	1.5 cm	---	0.2 cm	0.6 cm
Pseudomonas 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°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. **9_a** and **13_b** are exhibited the highest fungal inhibitory effect while **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.

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

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

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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