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Research Article

STRAIGHTFORWARD MULTICOMPONENT SYNTHESIS AND MICROBIAL SCREENING OF 2-CYANOAMINO-1, 2-DIHYDROPYRIMIDINES BEARING INDOLE SIDE CORE

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ABSTRACT

A new class of Indole connected cyanoaminopyrimidine scaffolds were synthesized by Multicomponent single-pot strategy with the reaction of indole-3-carbaldehyde, substituted acetophenone and cyanoguanidine in a basic alcoholic medium. The structural assignments were done for the obtained 2-Cyanoamino-6-aryl-4-(indol-3-yl)-1,2-dihydro-1H-pyrimidines (4a-4f) with the help of ^{1}H,^{13}C NMR, IR and Mass spectral data. The screened *in vitro* microbial activities of most of the compounds showed moderate to strong anti-microbial potential.

Keywords: Indole-3-carbaldehyde, Cyanoaminopyrimidine, Multicomponent synthesis, Cyanoguanidine, Microbial Screening.

1. INTRODUCTION

Broad spectrum of synthetic strategies and therapeutic utilities of azaheterocycles inspired the researchers to develop new methodologies as well as medicinally relevant drug targets. Such class of azaheterocycles, pyrimidine and aminopyrimidine derivatives relay wide range of biological activities such as Variolins B and D inhibits apoptosis by promoting neurite out growth in neuronal cell lines [1], urease inhibitors [2], anti-plasmodial [3] etc. and also present in different natural products such as vitamin-B_2 and folic acid [4] etc.

Cyanamide is a substle fragment to construct medicinally potent heterocycles [5] viz. 2-heteroimidazole, 2-aminosubstituted tetrazoles and oxazoles etc. explored significant biological activities [6], which are present in natural products [7]. Cyanoimines (=N-C≡N), cyanimides (RR'-N-C≡N) and cyanamides (RNH-C≡N) resembles with their structures and connected to other heterocyclic cores furnish biologically significant drug scaffolds and are considered as hypoglycaemic [8], antimycotic [9] etc.

Received therapeutic activities of cyanoiminopyrimidines like cytotoxic [10], antagonist [11] etc. emerged us to synthesis new class of cyanoiminopyrimidine with indolyl side core in a one pot methodology. Indole moiety bearing molecules exerts valuable biological activities [12], such as antipyretics, antitumor, anticonvulsant etc. Several natural products and drugs having indole core, act as non-selective COX inhibitors [13-14] (indo-methacin) and selective COX-2 inhibitors [15]. The introduction of heterocycles especially pyrimidine moiety at the third position of indole ring still enhance the medicinal value of the target molecules [16].

2. EXPERIMENTAL

Melting points were determined on a digital Buchi melting point apparatus and are uncorrected. Reagents and solvents were obtained from Sigma-Aldrich and E-Merck. Silica coated aluminium TLC plates were used to monitor the reaction progress and check the purity of compounds. The silica gel G (60-120 mesh) was used for column stationary phase. IR spectra were recorded on a Nicolet Avatar-360-IR spectrophotomer. The ^{1}H and ^{13}C NMR spectra were recorded using Bruker AMX 400 and 300MHz NMR spectrometer with TMS as internal standard and $CDCl_3$-d_6 as solvent. The mass spectra were recorded on an Agilent-GC 7890AMS5975C spectrometer.

2.1. Preparation of 2-Cyanoamino-6-aryl-4-(indole-3-yl)-1,2-dihydro-1H-pyrimidines(4a-4f)

The indole-3-carbaldehyde (10 mM), substituted aceto-phenone (10 mM) and cyanoguanidine (10 mM) in a

1:1:1 molar ratio were taken in a 100ml round bottomed flask containing sodium hydroxide (1g) in 95 percentage ethanol and refluxed for about three hours. The progress of the reaction was followed by thin layer chromatography. After completion of the reaction, the mass was cooled and the excess solvent was removed under reduced pressure. The final mass was filtered, dried and subjected to column chromatography purification (Benzene: Ethyl acetate as eluent).

2.1.1. 2-Cyanoamino-4-(indol-3-yl)-6-phenyl-1,2-dihydro-1H-pyrimidine(4a)

M.F:$C_{19}H_{14}N_5$, Melting point°C : 158,Yield %: 84; IR (cm^{-1}): 2185 (C≡N), 1636 (C=N), 3188 (N-H), 1531 (C=C); ^{1}H NMR, (δ,ppm): 4.37 (s,C_2-H), 5.01 (s, C_5-H), 6.37 (s,NH), 9.98 (s,NH, indole ring), 6.37-7.80 (m,NH and Aromatic); ^{13}C NMR (δ, ppm): 101.06 (5-C), 46.89 (2-C), 135.69(4-C), 155.67 (6-C) , 116.17 (C≡N) and 120.91-134.52 (Aromatic).

2.1.2. 2-Cyanoamino-6-(2,6-dichloro-4-methylphenyl)-4-(indol-3-yl)-1,2-dihydro-1H-pyrimidine (4b)

M.F:$C_{20}H_{14}N_5Cl_2$, Melting point°C :152,Yield %: 74; IR(cm^{-1}): 2183 (C≡N), 1635 (C=N), 3194 (N-H), 1532 (C=C); ^{1}H NMR, (δ,ppm): 4.36 (s,C_2-H), 4.97 (s, C_5-H), 6.21 (s,NH), 9.98(s,NH, indole ring), 2.30 (s,CH_3), 6.95-8.25 (m,NH and Aromatic); ^{13}C NMR (δ, ppm): 100.29 (5-C), 46.37 (2-C), 138.81 (4-C), 155.65 (6-C), 116.19 (C≡N), 23.39 (CH_3) and 120.93-134.47 (Aromatic).

2.1.3. 2-Cyanoamino-4-(indol-3-yl)-6-(4-methoxyphenyl)-1,2-dihydro-1H-pyrimidine(4c)

M.F:$C_{20}H_{16}N_5O$, Melting point°C :142,Yield %: 86; IR(cm^{-1}): 2185 (C≡N), 1640 (C=N), 3450 (N-H),1539 (C=C); ^{1}H NMR, (δ,ppm): 4.36 (s,C_2-H), 4.91 (s, C_5-H), 6.04 (s,NH), 10.00 (s,NH,indole ring), 3.76 (s,CH_3O), 6.81 -7.40 (m,NH and Aromatic); ^{13}C NMR (δ, ppm): 99.59 (5-C), 46.89 (2-C), 135.00 (4-C), 155.63(6-C), 113.45 (C≡N), 54.40 (CH_3O) and 120.91-132.38 (Aromatic).

2.1.4. 2-Cyanoamino-6-(4-bromophenyl)-4-(in-dol-3-yl)-1,2-dihydro-1H-pyrimidine(4d)

M.F:$C_{19}H_{13}N_5Br$, Melting point°C :116,Yield %: 84; IR(cm^{-1}): 2183 (C≡N), 1632 (C=N), 3448(N-H), 1534 (C=C); ^{1}H NMR, (δ,ppm): 4.37 (s,C_2-H), 5.01 (s, C_5-H), 6.01(s,NH), 10.01 (s,NH, indole ring), 6.93-8.06 (m,NH and Aromatic); ^{13}C NMR (δ, ppm): 101.13(5-C), 46.86 (2-C), 138.00(4-C), 155.70 (6-C), 116.06 (C≡N) and 120.00 -132.12 (Aromatic).

2.1.5. 2-Cyanoamino-6-(4-fluorophenyl)-4-(in-dol-3-yl)-1,2-dihydro-1H-pyrimidine(4e)

M.F:$C_{19}H_{13}N_5F$, Melting point°C : 138,Yield%: 80; IR(cm^{-1}):2185 (C≡N),1636 (C=N), 3188(N-H),1531 (C=C); ^{1}H NMR, (δ,ppm): 4.37(s,C_2-H), 4.94 (s,C_5-H), 6.45(s,NH), 10.07 (s,NH,indole ring),7.01-7.80(m, NH and Aromatic); ^{13}C NMR (δ, ppm): 105.75(5-C), 40.71(2-C), 134.60 (4-C),157.68 (6-C), 119.22(C≡N) and 124.97-130.94 (Aromatic).

2.1.6. 2-Cyanoamino-6-(4-chlorophenyl)-4-(in-dol-3-yl)-1,2-dihydro-1H-pyrimidine(4f)

M.F:$C_{19}H_{13}N_5Cl$, Melting point°C :114,Yield %: 88; IR(cm^{-1}): 2186 (C≡N), 1630 (C=N), 3445.3204 (N-H),1528 (C=C); ^{1}H NMR, (δ,ppm): 4.37 (s,C_2-H), 4.94 (s, C_5-H), 6.30 (s,NH), 10.00 (s,NH, indole ring), 7.08-7.33 (m,NH and Aromatic); ^{13}C NMR (δ, ppm): 101.54 (5-C), 46.92 (2-C), 134.92 (4-C), 155.72 (4-C), 118.30 (C≡N) and 125.48-132.09 (Aromatic).

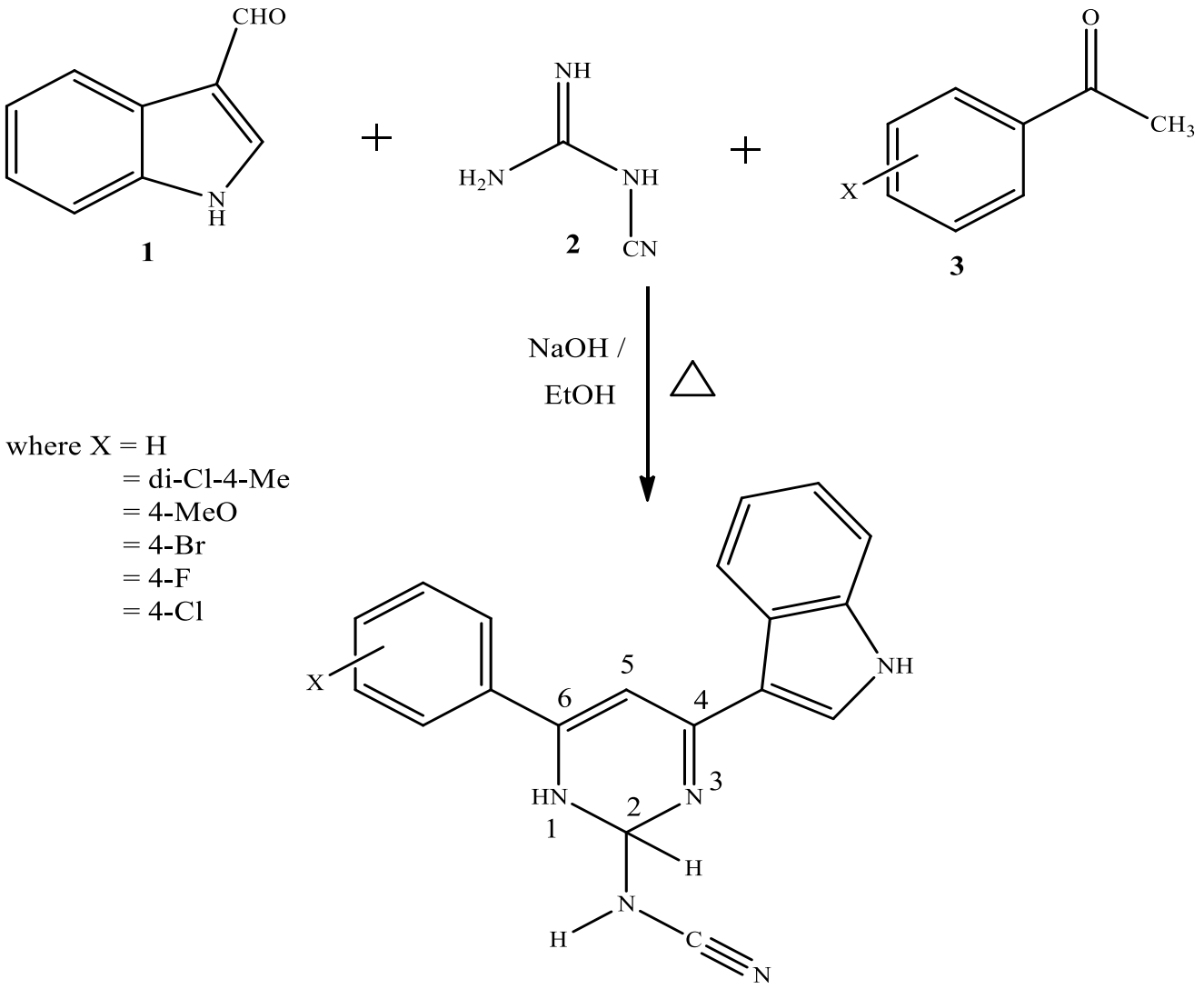
2.2. Antimicrobial Study

The microbial activity of synthesized compounds were examined against clinically isolated pathogens like gram negative bacterial strains such as *Escherichia coli* and *Klebsiella pneumonia* and gram positive *Staphylococcus aureus* and *Enterococcus faecalis* strains. In addition, the fungal strains viz. *Aspergillusflavus* and *penicillium chrysogenum* used to study the potency of the compounds. The drugs *Nitrofurantoin* and *Amphotericin- B* were used as the standards and Dimethyl Sulphoxide as the control.

3. RESULTS AND DISCUSSION

Steps reduced protocols in organic synthesis occupy a predominant place in research community. Diversity oriented drug lead synthesis through single step Multicomponent reaction (MCR) is a reliable and convenient method to the drug discovery offer outstanding value addition over linear multi-step synthesis [17-18]. The first reported multicomponent synthesis by Biginelli afforded dihydropyrimidines (DHPM) and continuing effort paid by the researchers to produce different functionalized dihydropyrimidines owing to the biological significance and pharmacological efficiency notably antihypertensive [19], adrenoceptor antagonists [20], antiproliferative and antitubilin activity [21] etc.

Our Multicomponent approach involves an exploratory reaction of indole-3-carbaldehyde (**1**), cyanoguanidine (**2**) and substituted acetophenone (**3**) using basic ethanolic medium yields the cyanoaminopyrimidines (**4a-f**) (Scheme-1).

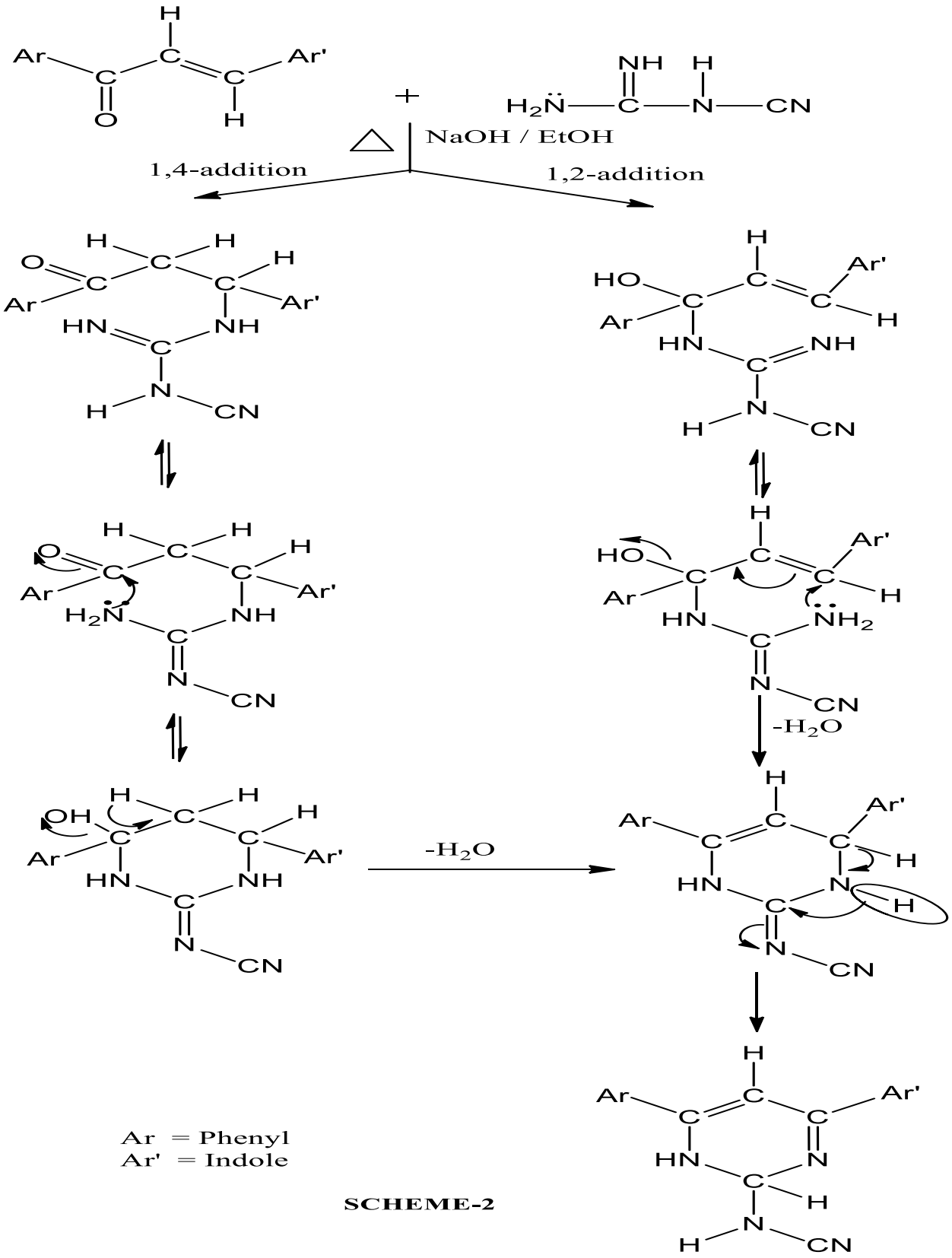


4a-f

SCHEME-1

An attempt is made to produce some indolyl based cyanoiminopyrimidines with the straight forward one-pot-startegy based on our previous reported multistep synthesis of cyanoiminopyrimidines [22] with different side chain functionalities. The unexpected 1,2-dihydrocyanoaminopyrimidine instead of 3,4-dihydro-cyanoiminopyrimidine obtained was characterized by NMR spectral chemical shift values with their multiplicity pattern. Encountered the reason for such result is based on the fact that cyanamides, cyanoimines and cyanoamines are structurally related and are exist as tautomers [23].

The ^{1}H NMR multiplicity pattern with chemical shift position of obtained compounds favours the structures resembles to cyanoamino-1,2-dihydropyrimidine. The mechanism to the formation of cyanoamino-1,2-dihydro-pyrimidine is expected to the following: 1,2-addition / 1,4-addition of cyanoguanidine on insitu formed indolyl strylphenylketone (chalcone) with subsequent cyclisation of the intermediate similar to the mechanism of 2-aminopyrimidine [24] and finally rearranges the hydrogen from cyanoimino-3,4-dihydropyrimidine afford cyano-amino-1,2-dihdro-pyrimidine (Scheme-2)



SCHEME-2

The ^{1}H NMR spectra of synthesized 1,2-dihydro-pyrimidines show two singlets in the aliphatic region between 4.3-5.01 ppm are the characterized singlets of H-2 and H-5 protons. One of the NH protons in the cyanoamino core gives the respective D_2O exchangeable singlet at around 6.2 ppm and one more NH proton merged in the aromatic region. The indole amino proton reflects in the chemical shift position at around

10.0 ppm as singlet. The aromatic protons show their corresponding multiplets in the region of 6.7-7.80 ppm. The Infra-Red bands in the stretching vibrational mode grants an inevitable evidences for the structural assignments of yielded pyrimidines. The IR spectra with an intense band at around 2180 cm^{-1} is due to the nitrile (C≡N) stretching vibrational mode. The absorption band appears at 3200-3400 cm^{-1} is responsible for NH stretching and the bands at ≈1530 and 1630 cm^{-1} corresponds to C=C and C=N stretching vibrations. The spectral data obtained from the IR and ^1H NMR sustains the assigned structure and the signals from carbon NMR spectra also enrich the structural assignment. Such that nitrile carbon resonates at around 116 ppm and the chemical shift values ≈ 46, ≈101, ≈135 and ≈155 ppm are corresponds to the carbons present in the position of 2C, 5C, 4C and 6C, respectively. The structures of the synthesized compounds are further confirmed by the analysis of mass spectrum of compound **4b** shows the corresponding M^{2+} peak.

3.1. Antimicrobial Study

The screened microbial activity results are listed in table 1. The data reveals that most of the compounds showed remarkable activity against tested gram positive bacteria especially against *Staphylococcus aureus*. The compounds exert moderate activity against tested gram negative bacterial strains. The compounds with higher antimicrobial potential against different pathogens are: unsubstituted(4a) and 4-fluro(4e) against *Escherichia coli;* 4-chloro (4f)and 4-methoxy (4c) against *Klebsiella pneumonia;* unsubstituted(4a),4-methoxy (4c) and 4-fluro(4e) against *Staphylococcus aureus* and unsustituted (4a) and 4-bromo(4d) against *Enterococcus faecalis.* The synthesized pyrimidines 4a, 4b, 4e and 4f are showed excellent anti fungal activity against the tested fungal strains notably 4a, 4b and 4f exerts higher active than the standards.

Table 1: Assay of antimicrobial activity

S.No	Microorganism	Zone of Inhibition (mm in diameter)							
	Bacteria	**Standard***	**Control**	**4a**	**4b**	**4c**	**4d**	**4e**	**4f**
1.	*Escherichia coli*	23	-	16	13	14	14	16	14
2.	*Klebsiella pneumoniae*	22	-	13	15	17	12	13	18
3.	*Staphylococcus aureus*	24	-	22	20	23	19	22	20
4.	*Enterococcus faecalis*	23	-	24	07	10	21	15	16
	Fungi								
5.	*Aspergillus flavus*	09	-	14	15	07	07	09	09
6.	*Penicillium chrysogenum*	13	-	13	14	-	-	07	15

**NIT-Nitrofurantoin (30 mcg), AP-Amphotericin-B*

4. CONCLUSION

The 2-Cyanoamino-6-aryl-4-(indole-3-yl)-1,2-dihydro-1H-pyrimidines were obtained from the One-Pot Multicomponent reaction of cyanoguanidine, substituted acetophenone and indole-3-carbaldehyde. The structures are assigned from the spectral data's and explored anti microbial study reveals the good microbial potential of synthesized compounds.

Conflict of interest

None declared

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