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Synthesis, Biological and Anti-Tumor Evaluation of Some New Nucleosides Incorporating Heterocyclic Moieties

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Abstract 1,3-diaryl-1-propen-3-ones **1a-h**, were used as building blocks for a large range of nucleoside analogs incorporating five and six-membered heterocyclic rings. Heterocyclic compounds incorporating aromatic moieties (**2-11**) and their *N*-nucleoside analogs (**13-20**) were synthesized. New compounds were evaluated for their potential antimicrobial and antifungal activities and for their *in vitro* cytotoxic activity against three cell lines: human breast cancer cell line (MCF-7), colon carcinoma cells (HCT) and human epidermid/arynx carcinoma cell line (HEp2).

Keywords Chalcones, Heterocyclic compounds, N-Nucleosides, Antimicrobial and anticancer activities

1. Introduction

Heterocyclic compounds occur widely in nature. Nitrogen-containing heterocyclic molecules constitute the largest portion of these chemical entities, which are part of many natural products. Indazole derivatives are interesting compounds, with many having biological as well as pharmaceutical activity [1-3]. Some new indazole derivatives were investigated as electronically active materials [4-9]. Cyanopyridone and cyanopyridine derivatives have promising antimicrobial activities [10, 11] as well as anti-cancer activities [12-14]. Oxazine derivatives represent an important classes of organic compounds; 1,3-oxazines in particular have been extensively studied because of their profound biological activities including antibacterial [15, 16], antifungal [17], antituberculor [18], antitumor and anti-HIV agents[19, 20]. 1,3-Oxazine derivatives are also known as progesterone receptor agonists [21]. Pyrimidine derivatives are very well known in medicinal chemistry for their therapeutic applications [22, 23]. One important class of pyrimidine is 2-thiopyrimidine and its derivatives, which are also well known as 2-mercaptopyrimidine compounds [24, 25]. Carbohydrates are ubiquitous in nature, readily available, cheap, biodegradable and non-toxic materials [26, 27]. Presence of several functional groups and stereogenic centers in carbohydrates permit stereochemical differentiations, enantiopure compound synthesis [28-30], use as chiral templates [31], biosensors [32] and as precursors for several biologically active products [33]. Besides these crucial roles, carbohydrates possess a unique set of chemical and structural feature that make them particularly attractive as molecular scaffolds.

The aim of this work was to design, synthesis of indazole, pyridines, 2-aminooxazines and/or 2-thiopyrimidine derivatives bearing aromatic moieties and use these compounds as a basis for the synthesis of a series of nucleosides. Some of the new compounds were then examined for cytotoxic activity via assays on human breast cancer cell line MCF-7, colon carcinoma cells (HCT), human epidermid/arynx carcinoma cell line (HEp2).

2. Results and Discussion

1,3-diaryl-1-propen-3-ones, **1a-h**, which were synthesized according to the literature [34], were used as starting material for the synthesis of a large range of heterocyclic compounds (compound series **2-6**) as depicted in Scheme 1.

Thus, condensation of **1h** with excess of hydrazine hydrate in dry ethanol led to the formation of the corresponding *N*-[4-(5-(4'-methoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl]phenyl)benzamide **2** [35-37]. The infrared spectrum of **2** showed absorption bands at 3340, 1650, 1600 due to υ_{NH}, $\upsilon_{C=O}$ (amide) and $\upsilon_{C=N}$, respectively. Compound **1f** reacted with ethyl acetoacetate (1:1) under the same conditions to afford ethyl 6-(2-chlorophenyl)-2-oxo-4-phenylcyclohex-3- ene carboxylate **3** [38-40] which reacted with hydrazine hydrate affording **4** [41]. The IR spectrum of **3** showed absorption bands at 1698, 1660 cm^{-1} due to two C=O groups, while its MS showed a molecular ion peak at m/z 355. The IR spectrum of **4** showed absorption bands at 3392 and 3216 for (NH/OH), 1670 (CONH) and 1604 (C=N). The mass spectrum of **4** revealed a molecular ion peak at m/z 322.

Reaction of **1c, f, g** with active methylene compounds

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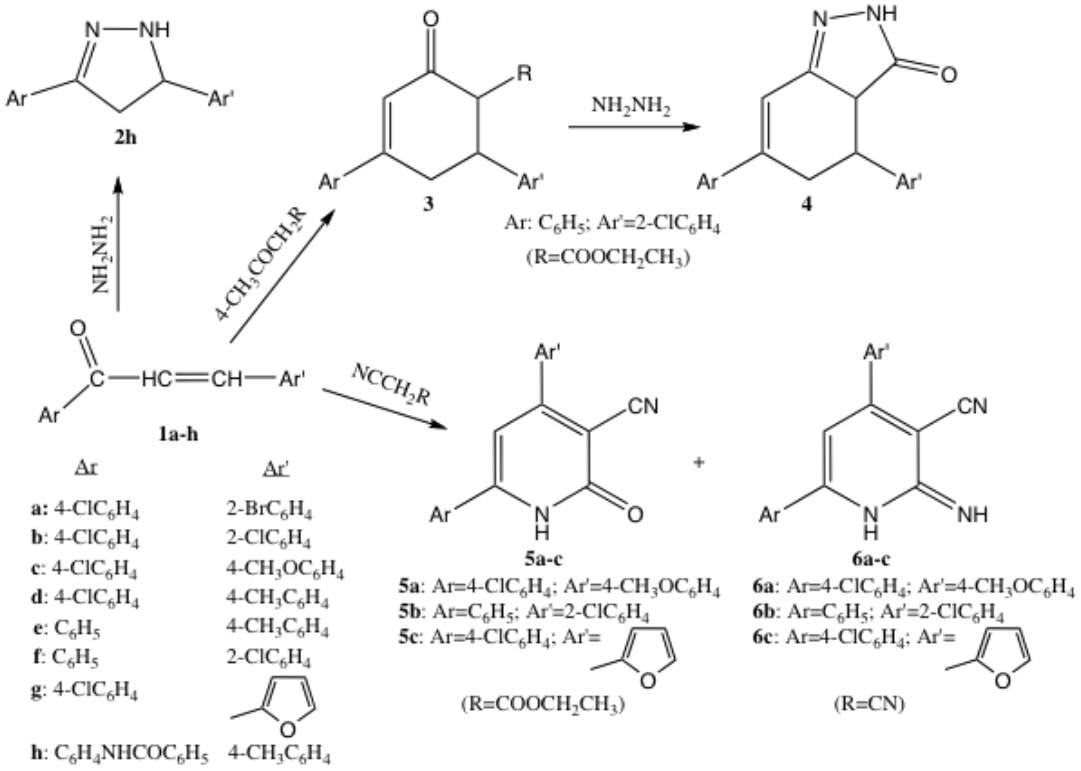
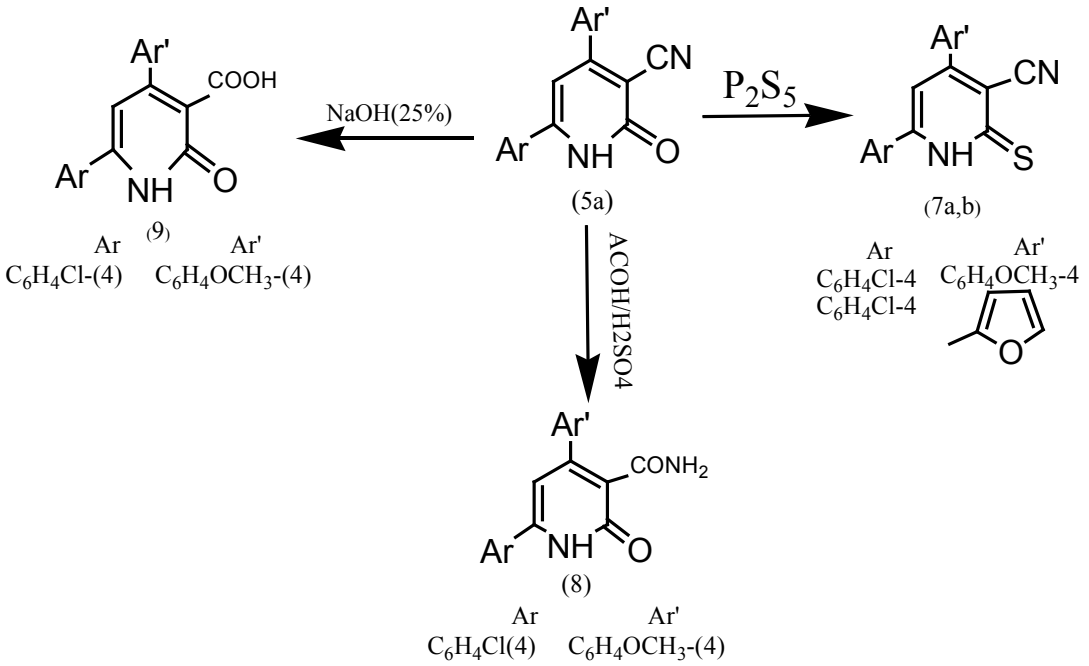
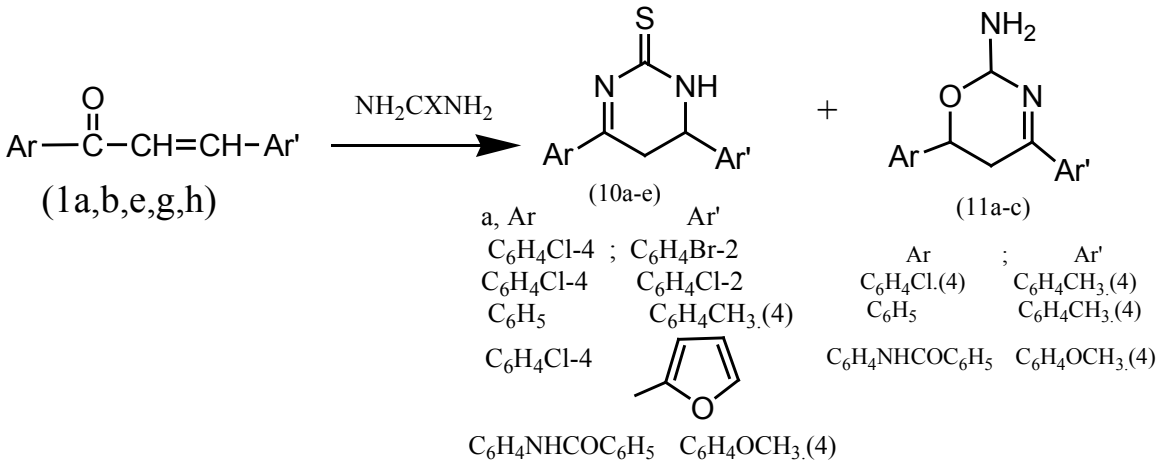
namely, ethyl cyanoacetate and /or malononitrile [42] in the presence of ammonium acetate afforded the corresponding 4,6-diaryl-2-oxo-1,2-dihydro-pyridine-3-carbonitrile **5a-c** and 2-imino-4,6-diaryl-1,2-dihydro-3-cabonitrile **6a-c**, respectively (Scheme1). The infrared spectra of **5a-c** showed absorption bands (in cm^{-1}) at 3460-3230 (NH/OH), 2230-2210 (C≡N) and 1654-1662 due to the amide C=O, while the infrared spectra of **6a-c** showed N-H absorption bands at 3366 and 3114 cm^{-1}, C≡N bands at 2220 and 2212 cm^{-1} and were devoid of $\upsilon_{C=O}$. The ^{1}H-NMR spectrum of **5a** (DMSO-d_6) showed signals at δ 3.83 ppm due to three OCH_3 protons, 8.28 ppm due to one NH proton and an aromatic multiplet at δ 7.12-8.00 ppm. Its MS spectrum showed a molecular ion peak at m/z 336.3.



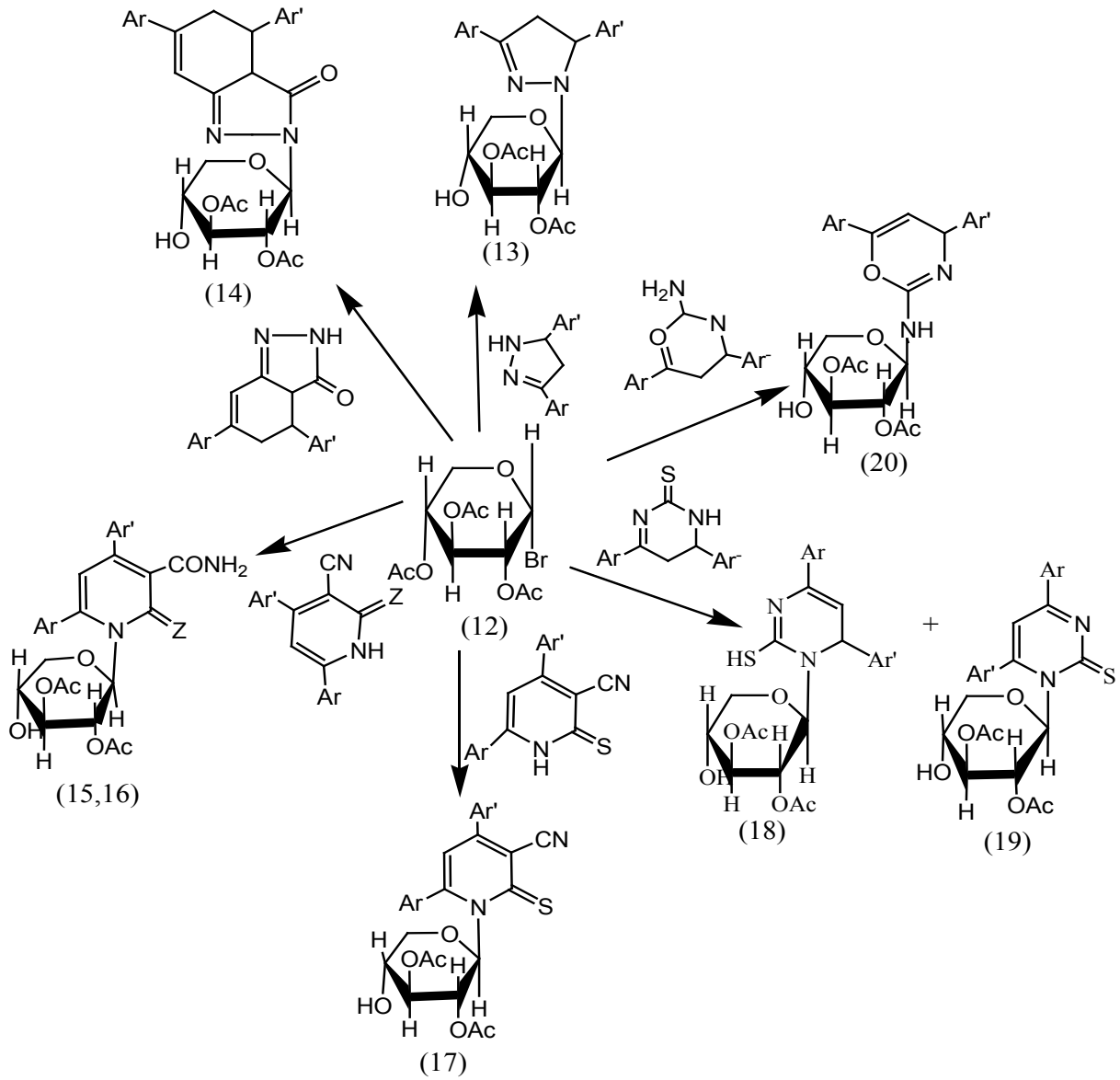
Scheme 1. Heterocycle synthesis from 1,3-diaryl-1-propen-3-ones



Scheme 2. Functionalizations of **5a**,**c**



Scheme 3. Heterocyclization reactions with **1a**,**b**,**e**,**g**,**h**



Scheme 4. Reaction of Compound **12** with Heterocycles

Scheme 2 depicts the reactions undertaken with cyanopyridone derivatives **5a**,**c.** The reaction of **5**a,**c** with phosphorus pentasulphide in a non-polar solvent, (e.g. xylene under reflux) afforded the corresponding 4,6-diaryl-2-thioxo-1,2-dihydropyridin-3-carbonitrile derivatives **7a-b**. The IR spectra showed absorption bands at 3354, 3437, 1218, 1212 cm^{-1} for NH and C=S, respectively. The mass spectrum of **7a** revealed a molecular ion peak at m/z 352.5. The reaction of compound **5a** with 40% H_2SO_4–AcOH afforded the corresponding 4,6-diaryl-2-oxo-1,2-dihydropyridine-3-carboxamide derivative **8a**. Its IR spectrum revealed absorption bands at 1648 due to the presence of C=O, NH/OH at 3410, 3464 cm^{-1} and were devoid of absorptions arising from the presence of C≡N. In a similar manner, hydrolysis of **5a** in ethanolic NaOH (25%) accompanied by oxidation with H_2O_2 afforded the corresponding 4,6-diaryl-2-oxo-1,2-dihydropyridin-3-carboxylic acid **9a**. Its IR spectrum revealed the presence of broad OH and NH absorption bands at 3433 and 3200 cm^{-1}, 1686 du, a strong C=O stretch and was devoid of any absorption for C≡N.

The reactions of 1,3-diaryl-2-propen-1-ones **1a,b,e,g** and **1h** with thiourea in boiling absolute ethanol containing sodium ethoxide afforded the corresponding 4,6-diaryl pyrmidine-2-thione derivatives **10a-e** in reasonable yields, while its reaction with urea under acid catalyzed conditions afforded the corresponding 2-amino-4,6-diaryl-1,3-oxazine derivatives **11a-c** respectively (Scheme 3).

The infrared spectra of **10a-e** displayed absorption bands at 3408-3140 cm^{-1}, 1112 and 1012 cm^{-1}, due to NH, and C=S, respectively. The ^{1}H-NMR spectrum of **10b** showed signals at δ 2.09 and 2.46 ppm due to two CH_2 protons, δ 7.82–8.12 ppm due to an aromatic multiplet and one D_2O exchangeable signal at δ 10.27 ppm due to an NH proton. The structure of **11a-c** was confirmed by infrared spectrum, which revealed the presence of N-H stretches at 3348-3200 cm^{-1}. The ^{1}H-NMR spectrum of **11b** showed signals at δ 2.1 ppm due to two NH_2 protons, δ 3.81 and 3.82 ppm due to a CHa proton, δ 6.9, 7.02 for a CHb proton and a multiplet for the aromatic protons at δ 7.5-8.1 ppm. The ^{13}C-NMR spectrum showed signals at δ 37.2(C_1), 161.1 (C_2), 97.07(C_3), 168.8 (C_4), 130.5 (C_5), 127.7 (C_6), 139.3 (C_7), 143.4 (C_8), 134.5 (C_9), 127.3 (C_{10}), 143.1 (C_1`), 129.6 ($C_3$`), 131.8 (C_6`), 128.3 ($C_5$`), 127.7 (C_2`), 119.4 ($C_4$`) and its mass spectrum showed a molecular ion peak m/z at 284.8.

Interaction of α-D-xylopyranosyl bromide **12** with the desired heterocycles in aqueous potassium hydroxide afforded the corresponding heterocycles incorporating tetrahydro-2H-pyran-3,4-diyldiacetates (**13-20**). See Scheme 4.

Reaction of compound **2** with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide **12** gave the corresponding **13**. Its infrared spectrum revealed the presence of absorption bands at 1663, 1600, and 3346 cm^{-1} due to CONH and NH/OH respectively. The ^{1}H-NMR (DMSO-d_6) of **13** showed a doublet for anomeric protons at δ 6.9, 7.0 ppm due to diaxial orientations of H-1' and H-2' protons, indicating their presence in the β-configuration and the other protons of the xylopyranosyl resonating in the region δ 3.3-3.7 ppm, while the protons of the two acetyl moieties showed as two singlets at δ 2.41 and 2.43 ppm. The presence of the two OH protons was indicated by two singlets at δ 10.04 and 10.52 ppm. The mass spectrum of **13** showed a molecular ion peak at m/z 575 indicating the partial hydrolysis of one acetyl and the metonym groups in the molecule to the corresponding OH group.

Similarly α-D-xylopyranosyl bromide **12** was reacted with the indazolone **4** under the same conditions to give the corresponding (2R,3S,4R,5S)-5-hydroxy-2-[3-oxo-(4-phenyl-5-(2-chlorophenyl)-3,3a,4,5-tetrahydro-2H-indazol-2-yl)tetrahydro-2H-pyran-3,4-diyl diacetate **14.** Its IR spectrum revealed the presence of absorption bands at 3443 cm^{-1} (OH), 1742 cm^{-1} (C=O) and an absorption band at 1376 cm^{-1} due to an out of plane CH_3. On the other hand, 2, 3, 4, 6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide **12** reacted with **5c** and **6c** in acetone and aqueous potassium hydroxide to afford the corresponding nucleosides derivatives **15** and **16**, respectively. The IR spectra of **15** and **16** showed absorption bands at 3315-3314 cm^{-1} (br) and 1655-1648 cm^{-1} due to OH/NH and C=O groups, respectively. Spectral results showed that the electron withdrawing character of 4-chlorophenyl and the furan ring at positions 4 and 6 in the pyridine ring of the nucleosides **15** and **16** were helpful in the partial hydrolysis of the cyan group at position 3 of the pyridine moiety as evidenced by the fact that no cyano stretching absorption band was observed in the IR. The ^{1}H–NMR spectrum of **15** (DMSO-d_6) showed signals at δ 4.41 ppm due to NH protons, an aromatic multiplet at δ 6.68-7.90 ppm, two doublets at δ 8.05 and 8.07 ppm due to OH protons, triplets at δ 3.3, 3.41, 3.44 ppm due to CH_2 protons and a singlet at δ 8.07 ppm due to NH_2 protons. Its mass spectrum showed a molecular ion peak at m/z 497.45. The ^{1}H-NMR spectrum of **16** (DMSO-d_6) showed signals at δ1.97 and 2.49 ppm as two singlets arising from the two $COCH_3$ groups at $C_2^{'}$, $C_3^{'}$, a singlet signal at δ 6.55 ppm due to proton in the pyridine moiety, two doublets at δ 2.44 and 3.44 ppm due to two anomeric protons at $C_2^{'}$ and $C_3^{'}$, a multiplet at δ 6.82-7.94 ppm for the aromatic protons and two signals, one at δ 8.12 ppm for the NH_2 and at δ 9.75 ppm due to the OH proton at $C_4^{'}$. Its mass spectrum showed the molecular ion peak at m/z 511.5.

The reaction of 2,3,4-tri-O-acetyl-α-D-xylopyranosyl bromide **12** with 4,6-diaryl-3-cyano-2-thioxo-1-(2H)-pyridines **7a** and **b** afforded the corresponding 2R,3S,4R, 5S-2-(4,6-diaryl-3-carbonitrile)-2-thioxo-1(2H)-pyridin-yl)-5'-hydroxy-tetrahydro-2H-pyran-3,4-diyl) diacetates **17a** and **b**, respectively. This is supported by the presence of C≡N stretching absorptions at 2220 and 2228 cm^{-1} and 1248 and 1232 cm^{-1} for the C=S moiety. The mass spectrum of **17a** showed molecular ion peak at m/z 351.5. The electron releasing effect of OCH_3 at position-4 of the aromatic moiety was balanced by the electron withdrawing effect of the chlorophenyl, thereby stabilizing the cyano group at position 3 towards alkaline hydrolysis. The electronic character of the sulphur moiety at carbon 2 may also have helped stabilize the

–C≡N group towards the effect of the alkali on the compound **17b**. Interaction of 2,3,4-tri-O-acetyl-α-D- xylopyranosyl bromide **12** with pyrimidine thione derivatives **10a** and **10c** in aqueous KOH/acetone afforded the corresponding 2S,3S,4R,5S-2-(6-(phenyl)-4-(2-bromo phenyl)-2,3,4,5-tetrahydro-pyrimidin-1-yl)-mercapto-5-hydroxy tetra hydro-2H-pyran-3,4-diyl)diacetate **(18a)** and 2S, 3S,4R,5S-2-(6-(4-chloro phenyl)-4-(2-bromo phenyl)-2, 3,4,5-tetrahydro-pyrimidin-1-yl)mercapto-5-hydroxytetrahydro-2 H-pyran-3,4-diyl)diacetate (**18b**), respectively. The IR spectra of **18a** and **18b** revealed weak absorption bands arising from SH stretching at 2652 and 2644 cm^{-1} and were devoid of C=S absorptions. The ^{1}H-NMR spectrum of **18a** (DMSO-d_6) showed signals at δ 1.19 ppm, 2.49 ppm ($COCH_3$ protons), δ 3.32 ppm due to CH_2 protons, δ 4.29 ppm (CH proton), a multiplet at δ 7.23-7.64 ppm, δ 9.05 (SH proton) and at δ 9.99 ppm due to OH proton. The mass spectrum of **18b** showed a molecular ion peak at m/z 424.

Furthermore, 2,3,4-tri-O-acetyl-α-D-xylopyranosyl bromide **12** reacted with other pyrimidine thione derivatives **10b,d** and **e** in aqueous KOH/acetone to afford the corresponding nucleosides **19a-c**. The infrared spectra of **19a-c** revealed the presence of absorption bands at 3401, 3343, 3294, 1740, 1674, 1670, 1256-1240 cm^{-1} for OH/NH, C=O and C=S functionalities . The ^{1}H-NMR spectrum of **19a** (DMSO-d_6) showed signals at δ 2.49 and 2.50 ppm due to CH_2 protons, δ 5.39 and 5.40 ppm due to three CH_3 protons, and at δ 10.06 ppm due to OH protons, while its MS showed a molecular ion peak at m/z 300. The^{1}H-NMR spectrum of **20c** (DMSO-d_6) showed signals at δ 3.55 ppm due to three OCH_3 protons and δ 5.93 ppm due to NHCO proton. Interaction of 2-amino-4,6-diaryl-1,3-oxazine derivatives **11b** and **11c** with 2,3,4-tri-O-acetyl-α,D-xylo pyranosyl bromide **12** afforded the corresponding nucleosides **20a** and **20b**, respectively. The infrared spectra of **20a** and **20b** revealed absorption bands at 3482, 3302, 1654, 1666 cm^{-1}, due to OH/NH and C=O groups. The ^{1}H-NMR (DMSO-d_6) of **20a** showed singlet signals at δ 2.49 and 2.495 ppm due to two acetyl groups, a singlet at δ 3.91 ppm due to pyran CH_2 units, a doublet at δ 4.0 ppm (oxazine ring CH – CH), a singlet at δ 4.06 ppm due to NH proton, a multiplet due to the aromatic protons at δ 7.42–7.82 ppm and a singlet at δ 8.05 ppm due to OH protons. Its mass spectrum showed a molecular ion at m/z 484.5. The ^{1}H-NMR (DMSO-d_6) of **20b** showed a doublet at δ 6.9 ppm due to anomeric protons of C–1', C–2' indicating the presence of a β configuration and the other protons of the xylopyranozyl at δ 2.4–2.5 ppm. The acetyl protons showed two singlets at δ 2.1 ppm and 2.6 ppm, the NH proton showed a singlet at δ 10.2 ppm. The OH proton of $C_{4'}$ resonated at δ 10.5 ppm, three protons of the methoxy group showed a singlet at δ 3.8 ppm, while the aromatic protons gave a multiplet at δ 7.02–8.13 ppm. Its ^{13}C-NMR (DMSO-d_6) showed two signals for the $COCH_3$ groups at δ 24.1 and 26.4 ppm, a signal at δ 38.6 ppm due to OCH_3 and a methylene group signal at δ 55.3 ppm.

3. Biological Evaluation

3.1. Antimicrobial Activity

Previously untested compounds were evaluated for antimicrobial activity against eight strains of microorganisms using the agar diffusion technique. The tested compounds were screened against two Gram-positive bacteria, Staphylococcus aureus (RCMB 000106) Bacillus subtilis (RCMB 000107); two Gram-negative bacteria, Pseudomonas aeruginosa (RCMB 000102), Escherichia coli (RCMB 000103) and four fungi, Aspergillus fumigatus (RCMB 002003), Geotrichum candidum (RCMB 052006), Candida albicans (RCMB 005002) and Syncephalastrum racemosum (RCMB 005003) by the disk diffusion method. Penicillin G. Streptomycin were used as positive control for bacterial strains while, Itraconazole and Clotrimazole were used as positive controls for the fungi strains. The investigation of antibacterial screening data revealed that compounds **5c** and **10e** were the most potent towards the Gram-positive bacteria S. aureus and B. subtilis. Compounds **6c, 10e** and **11b** showed good to moderate activity against Gram-positive bacteria S. aureus and B. subtilis. Compound **2** was less potent, while **7b, 10a** were inactive. As for the bacterial inhibition of the Gram-negative bacteria, the screening data showed that compounds, **5c** and **7a** were the most potent against E. coli. Compounds **2, 6c** and **11b** showed a relatively poor inhibition towards E. coli. All the tested analogs showed no activity against P. aeruginosa. Similarly, compounds **7b** and **10a,c**,e were inactive against the Gram-negative bacteria P. aeruginosa or E. coli. The bacterial zone of inhibition values are given in Table **1**.

Minimum inhibitory concentrations (MICs) were determined by the broth dilution technique. The nutrient broth, which contained logarithmic serially two fold diluted amounts of test compound, and controls were inoculated with approximately 5 x 10^5 c.f.u./ml of actively dividing bacteria cells. The cultures were incubated for 24h. At 37°C and the growth was monitored visually and spectrophotometrically. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as minimum inhibitory concentration (MIC). To obtain the minimum bactericidal concentration (MBC), 0.1 ml volume was taken from each tube and spread on agar plates. The number of c.f.u. was counted after 18-24 hrs. of incubation at 35°C. MBC was defined as the lowest drug concentration at which 99.9% of the inoculums were killed. The minimum inhibitory concentration and minimum bactericidal concentration are given in Table 2.

Table 1. Antibacterial activity of compounds 2, 5c, 6c, 7a,b, 10a,c,e and 11b

Comp. No.	Diameter of zone of inhibition (mm)			
	Gram-positive bacteria		Gram-negative bacteria	
	S. aureus	***B. subtilis***	***P. aeruginose***	***E. coli***
2	17.9±0.05	16.1±0.01	NA	10.1±0.01
5c	24.0±0.01	26.4±0.05	NA	18.8±0.02
6c	20.0±0.08	19.5±0.03	NA	9.8±0.06
7a	23.4±0.01	25.4±0.03	NA	16.3±0.08
7b	NA	NA	NA	NA
10a	NA	NA	NA	NA
10c	NA	NA	NA	NA
10e	20.4±0.08	21.8±0.01	NA	NA
11b	21.2±0.05	22.8±0.09	NA	9.6±0.08
Standard a	29.48±0.82	32.56±0.5	28.32±0.1	33.56±0.07
Standard b	25.0±0.2	29.0±0.04	24.0±0.1	25.0±0.03
DMSO	--	--	--	--

Positive control (standards, a, b): Penicillin G and streptomycin and negative control DMSO measured by the Halo zone test (unit, mm).

Table 2. MIC and MBC results of compounds 5c, 6c, 7a and 11b

Comp. No.	Diameter of zone of inhibition (mm)							
	Gram-positive bacteria				Gram-negative bacteria			
	S. aureus		B. subtilis		P. aeruginose		E. coli	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
5c	39	100	19	50	NA	>100	156	>100
6c	78	>100	156	>100	NA	>100	625	>100
7a	39	100	19	50	NA	>100	156	>100
11b	39	100	39	100	NA	>100	625	>100

MIC (μg/ml) = minimum inhibitory concentration, that is, the lowest concentration of the compound to inhibit the growth of bacteria completely; MBC (μg/ml) = minimum bactericidal concentration, that is the lowest concentration of the compound for killing the bacteria completely.

Table 3. MIC* and MFC of compounds 5c, 6c, 7a, 11b

Comp. No.	Diameter of zone of inhibition (mm)							
	AF		***GC***		***CA***		***SR***	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
5c	39	78	19	39	78	156	156	313
6c	39	78	39	78	156	313	313	625
7a	19	39	19	39	78	156	156	313
11b	39	78	78	156	156	313	313	625

* Positive control Intraconazole and clotrimazole. AF: *Aspergillus fumigatus*, GC: *Geotrichum candidum*, CA; *Candida albicans*, SR: *Syncephalastrum racemosum.* MIC (μg/ml) = minimum inhibitory concentration, that is the lowest concentration of the compound to inhibit the growth of fungus completely. MFC (μg/ml) = minimum fungicidal concentration, that is, the lowest concentration of the compound for killing the fungus completely.

3.2. Antifungal Studies

Antifungal activity testing was also done by the disk diffusion method [43]. For assaying antifungal activity Aspergillus fumigatus (RCMB 002003), Geotrichum candidum (RCMB 052006), Candida albicans (RCMB 005002) and Syncephalastrum racemosum (RCMB 005003) were recultured in DMSO by the agar diffusion method. The lowest concentration (highest dilution) required to arrest the growth of fungus was regarded as minimum inhibitory concentration (MIC). The minimum inhibitory concentration and minimum fungicidal concentration are given in Table 3.

According to the results of bioactivity studies, it is noted that compounds **5c** and **7a**, which contain the pyridine-3-carbonitrile moiety, provide better antimicrobial activity against S. aureus and B. subtilis than the other compounds **(6c**, **10e**, **11b).** Surprisingly, the pyrimidine thione derivatives (**10a, c**) were inactive against any

Gram-positive bacteria, Gram-negative bacteria or fungi. Compounds **(5c, 6c, 7a)** which contain 2-thioxo-3-carbonitrile pyridine moiety, 2-amino-3-carbonitrile pyridine moiety and pyridone-3-carbonitrile moiety, respectively, exhibited the best antimicrobial activity against the fungi tested.

3.3. Cytotoxicity Studies

Cytotoxicity tests were performed using compounds **4**, **5c**, **10c** and **11c** against three cancer cell lines, breast cancer cell line MCF-7, colon carcinoma cells (HCT), human epidermid/arynx carcinoma cell line (HEp2) by using a modified method [35]. The results (Table **4**) showed that **4** had slight activity toward the HCT cell line (IC_{50} = 4.7 µg/ml) and its activity towards MCF-7 cell line was lower (IC_{50} = 2.7 µg/mL). Compound **11c** exhibited cytotoxic activity against the HCT cell line (IC_{50} 10.2 µg/ml) and a higher cytotoxic activity against MCF-7 with IC_{50} = 20.7 µg/ml. The cytotoxic activity of **5c** towards HEP-2 was moderately potent with an IC_{50} = 10.2 µg/ml while its cytotoxic activity against colon carcinoma cells was very low with an IC_{50} = 2.1µg/ml. The cytotoxic activity of **10c** towards the MCF-7 cell line was relatively weak with an IC_{50} = 4.8 µg/ml, while the cytotoxicity against HCT cell line was nearly inactive given the observed IC_{50} of 0.5 µg/ml.

Table 4. In vitro Cytotoxic activity of 4, 5c, 10c, 11c in human MCF-7, HCT, HEp2 cell lines

Cell lines[a]	IC_{50} (µg/ml)[b,c]			
	4	**5c**	**10c**	**11c**
MCF-7	2.7	--	4.8	20.7
HCT	4.7	2.1	0.5	10.2
HEp2	--	10.2	--	--

[a] Cancer cell lines were breast carcinoma cells (MCF-7), colon carcinoma cells (HCT), human epidermid/arynx carcinoma cell line (HEp2). [b] The assays were performed in triplicate.

Table 5. In vitro Cytotoxic activity of nucleoside analogs 14, 20b, 17a, 18a in human MCF-7, HCT, HEp2 cell lines

Cell lines[a]	IC_{50} (µg/ml)[b]			
	14	**17a**	**18a**	**20b**
HCT	0.9	NT	19.2	16.7
MCF-7	1.5	NT	1.1	14.4
HepG2	NT	2	NT	NT

[a]Cancer cell lines were colon carcinoma cells HCT, Hepatocellular, NT indicates not tested.
[b]Assays were performed in triplicate. carcinoma cells HepG2, Breast carcinoma cells MCF-7.

The nucleoside analogs **14**, **17a**, **18a**, and **20b** (Table 5) showed cytotoxic activity against HTC and MCF-7 and Hepatocellular carcinoma cells HepG2. The IC_{50} of compound **14,** with values 0.9 and 1.5 µg/ml, indicates high potency against HCT and MCF-7, respectively [44, 45]. The nucleoside analog **17a** exerted cytotoxic activity against $HepG_2$ with IC_{50} = 2 µg/ml. The nucleoside analog **18a** exerted activity against HCT with IC_{50} of 19.2 µg/ml which decreased when tested against MCF-7 to an IC_{50} = 1.1 µg/ml. The nucleoside analog **20b** selectively exhibited cytotoxic activity against HCT and MCF-7 cell lines with IC_{50} of 16.7 and 14.4 µg/ml respectively.

4. Conclusions

In summary, we have synthesized a novel series of nucleoside analogs in moderate to high yields. The prepared compounds which contain the pyridine-3-carbonitrile moiety provide better antimicrobial activity against *S. aureus, B. subtilis* than similar molecules without this functionality. Most of the prepared compounds revealed potential anticancer activity against the colon cancer cell line, Hepatocellular cancer cell line, Breast cell line and epidermid / arynx cancer cell line. Compounds **4**, **5c**, **10c**, **11c**, **14**, **17a**, **18a** and **20b** exhibited good antitumor activity when compared with the reference drug.

5. Experimental

All melting points for the prepared derivatives were measured in capillary tubes using a Gallen-Kamp apparatus and were uncorrected. The IR spectra were recorded on a Perkin-Elmer 1650 spectrophotometer (KBr pellets) and the wave numbers were given in cm^{-1}. The ^1H, ^{13}C NMR spectra were measured in dimethyl sulphoxide-d6 as a solvent using a Varian Gemini 180 spectrometer operating at 300 MHz for ^1H, and 75 MHz for ^{13}C. TMS was used as an internal standard and the chemical shifts were reported as δ ppm. The FAB mass spectra were recorded on a JEOL SX 102/DA-6000 mass spectrometer.

Synthesis of N-(4-(5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl)benzamide (2).

A mixture of (E)-N-(4-(3-(4`-methoxyphenyl) acryloyl) phenyl) benzamide **(1h)** (0.01mol) and hydrazine hydrate (0.01mol) in 30 ml of ethanol was refluxed for 6h. The yellow precipitate formed after cooling was filtered off, dried and recrystallized from ethanol to afford the required product **(2)** as yellow crystals, in 75% yield, m.p. 181°C; [Requires: C, 74.39; H, 5.66; N, 11.32; Found: C, 74.35; H, 5.7; N, 11.4. IR (cm^{-1}): 3340, 2942, 2846, 1650, 1600, 1510.

Synthesis of ethyl 6-(4-chlorophenyl)-2-oxo-4-phenylcyclohex-3-ene carboxylate (3).

A mixture of **(1f)** (0.01mol) and ethylacetoacetate (0.01mol) in 30ml of absolute ethanol containing sodium ethoxide (prepared from 0.2 g of sodium metal and 4.6 ml of absolute ethanol) was refluxed for 6h. After concentration and cooling the residue was poured into water, filtered off, washed well with dilute alcohol and recrystallized from ethanol to afford **3** as white crystals, in 60% yield, m.p 124°C. Requires: C, 71.08; H, 5.35; Cl, 10.01: Found: C, 71.1; H, 5.4; Cl, 10.1. IR (cm^{-1}): 3006, 2928, 2818, 1698, 1660. MS (*m/z*, %); 355 (9.2%), 320 (3.8%), 308 (6.1%), 278 (34.6%), 249(1.6%), 192 (4.8%), 144 (100%).

Synthesis of 4-(4-chlorophenyl)-6-phenyl-3,3a,4,5-tetrahydro-2H-indazolone (4).

A mixture of **3** (0.01 mol) and hydrazine hydrate (0.01 mol) in 15 ml of acetic acid was heated under reflux for 6h. The solvent was evaporated and the product was collected, washed well with dilute ethanol and recrystallized from ethanol to give **(4)** as brown crystals, in 60% yield, m.p 144°C. Requires: C, 70.69; H, 4.65; N, 8.68; Cl, 11.007: Found: C, 71.0; H, 4.7; N, 8.7; Cl, 11.1IR (cm^{-1}): 3392, 3216; 2918, 2846, 1670, 1604, 1508. MS (*m/z*, %); M^+, M^{+2}, M^{+3}, 322, 324, 325 (99.4, 30.7, 4.6%).

Synthesis of 4,6-diaryl-2-oxo-1,2-dihydropyridine-3-carbonitrile (5a-c).

A mixture of **1c**, **1f**, and **1g** (0.01 mol), ethylcyanoacetate (0.01 mol) and ammonium acetate (0.04 mol) in 30 ml of absolute ethanol was heated under reflux for 6h. It was then allowed to cool, filtered off, washed well with water, then with dilute alcohol, and recrystallized from ethanol to give the corresponding derivatives **(5a-c)** respectively.

5a as yellow crystals, in 70% yield, m.p. 51°C. Requires: C, 67.75; H, 3.86; N, 8.32; Cl, 10.54: Found C, 67.6; H, 3.9; N, 8.4; Cl, 10.2; MS (*m/z*, %); 336.3 (96.0%) M^+, 50 (100%). ^{1}H-NMR spectrum (DMSO-d_6): δ 3.83 (s, 3H, OCH_3), 7.12-8.10 (m, 8H,C=CH and Ar-H) 8.28 (s, 1H, NH).

5b as white crystals, in 70% yield, m.p. 80°C. Requires: C, 70.47; H, 3.58; N, 9.13; Cl, 11.58: Found C, 70.5; H, 3.6; N, 9.2; Cl, 11.6; IR (cm^{-1}): 3460-3230, 2230-2210, 1654-1662.

5c as brown crystals, in 75% yield, m.p. 60°C. Requires: C, 64.75; H, 3.03; N, 9.44; Cl, 11.97: Found: C, 64.5; H, 3.00; N, 9.5; Cl, 11.5; IR (cm^{-1}): 3460-3230, 2230-2210, 1654-1662.

Synthesis of 2-imino-4,6-diaryl-1,2-dihydropyridin-3-carbonitrile (6a-c).

A mixture of **1c**, **1f**, and **1g** (0.01 mol), malononitrile (0.01 mol) and ammonium acetate (0.04 mol) was fused on a sand-bath at 135-165°C for 3h. The product formed after cooling was washed with water, then with dilute ethanol, and recrystallized from the proper solvent to give **(6a-c)** respectively.

6a as white crystals, in 71% yield, m.p. 52°C. Requires: C, 67.95; H, 4.17; N, 12.51; Cl, 10.58: Found: C, 68.0; H, 4.2; N, 9.5; Cl, 12.5; Cl, 10.6; IR (cm^{-1}): 3100, 2220, 1662, 690 (C–Cl).

6b as white crystals, in 70% yield, m.p. 75°C. Requires: C, 70.70; 3.92; N, 13.74; Cl, 11.62: Found: C, 70.7; H, 3.9; N, 13.8; Cl, 11.6; IR (cm^{-1}): 3366, 2212, 1626, 686 (C–Cl).

6c as black crystals in 60% yield, m.p. 70°C. Requires: C, 64.67; 3.38; N, 14.21; Cl, 12.01: Found: C, 65.0; H, 3.4; N, 4.1; Cl, 12.1; IR (cm^{-1}): 3114, 2208, 1648, 640 (C–Cl).

Synthesis of 4,6-diaryl-2-thioxo-1,2-dihydropyridin-3-carbonitrile derivatives (7a,b).

A mixture of **5a** and/or **5c** (0.01mole) and P_2S_5 (0.01mol) in 15ml of dry xylene was refluxed for 6h. After cooling the solvent was evaporated under reduced pressure and the product was treated with petroleum ether (b.p. 40-60°C), then recrystallized from the proper solvent as **7a,b**.

7a as reddish brown crystals, in 70% yield, m.p. 200°C. Requires: C, 64.68; H, 3.68; N, 7.94; S, 9.07; Cl, 10.07: Found: C, 64.7; H, 3.7; N, 7.9; S, 9.1; Cl, 10.1; IR (cm^{-1}): 3354, 3437, 1590, 1588, 1218, 1212. MS *m/z*, (%): 352.5 (9.82%), 324.2 (9.57%), 270 (10.18%), 241.2 (9.1%, 207.2 (10.55%), 171.2 (9.38%), 63 (100%).

7b as dark brown crystals, in 60% yield, m.p. 110-112°C. Requires: C, 61.44; H, 2.88; N, 8.96; S, 10.24; Cl, 11.36: Found: C, 61.5; H, 2.9; N, 8.8; S, 10.2; Cl, 11.4; IR (cm^{-1}): 3354, 3437, 1590, 1588, 1218, 1212.

Synthesis of 6-(4-chlorophenyl)-4-(4-methoxyph-enyl)-2-oxo-1,2-dihydropyridin-3-carboxamide (8)

A mixture of **(5a)** (1g) of and (5ml) of 30-40% H_2SO_4 in 15ml acetic acid was refluxed for 6hrs. The Precipitate was filtered off and recrystallized from benzene to give **(8)** as white crystals in 60% yield, m.p 120°C. Requires: C, 67.35; H, 4.43; N, 8.27; Cl, 10.48: Found: C, 67.1; H, 4.1; N, 8.1; Cl, 10.23; IR (cm^{-1}): 3410, 3464, 1648.

Synthesis of 6-(4-chlorophenyl)-4-(4-methoxyph-enyl)-2-oxo-1,2-dihydropyridine-3-carboxylic acid (9).

A mixture of **(5a)** (0.2 mol), ethyl alcohol (13 ml) and (3 ml) of 25% NaOH was stirred on magnetic stirrer, (10 ml) of 30% H_2O_2 was added gradually, the solution left to cool in ice bath. After an hour, the reaction was permitted to run at 50°C for an additional 3h. Then 5% sulphuric acid was added to neutralize the solution, the solvent was evaporated and solid product was recrystallized from benzene to give **9** as white crystals in 60% yield, m.p. 170°C. Requires: 67.16; H, 4.12; N, 4.12; Cl, 10.45: Found: C, 67.1; H, 4.1; N, 4.1; Cl, 10.23; IR (cm^{-1}): 3433, 3200, 1686, 1590.

Synthesis 4,6-diaryl-5,6-dihydropyrimidin-2(1H)-thione derivatives (10a-e).

A mixture of **(1a,b,f, g,** and **h)** (0.01 mol) and thiourea (0.01mol) in 30ml ethanol containing (0.01mol) sodium ethoxide was heated under reflux for 6h. After concentration and cooling, the residue was diluted with water, filtered off, then washed well with warm water and dilute alcohol and recrystallised from the proper solvent to give **(10a-e).**

10a as pale white crystals in 70% yield, m.p. 200°C. Requires: C, 50.59; H, 3.16; N, 7.37; S, 8.43; Cl, 9.35; Br, 21.08: Found: C, 50.6; H, 3.2; N, 7.4; S, 8.4; Cl, 9.4; Br, 21.1; IR (cm^{-1}): 3408, 3156, 2620, 1662, 1014, 454, 580 (C-Br, C-Cl).

10b as brown crystals in 70% yield, m.p. 190°C. Requires: C, 57.31; H, 3.58; N, 8.35; S, 9.55; Cl, 21.19; Found: C, 57.3; H, 3.6; N, 8.4; S, 9.6; Cl,21.2; IR (cm^{-1}): 3398, 3152, 2620, 1656, 1018, 580 (C-Cl).

10c as yellow crystals in 70% yield., m.p. 135°C. Requires: C, 55.65; H, 3.76; N, 8.11; S, 9.27; Br, 23.18; Found: C, 55.7; H, 3.8; N, 8.2; S, 9.3; Br, 23.3; IR (cm^{-1}): 3325, 3150, 2620, 1650, 1018, 580 (C-Cl).

10d as dark brown crystals in 60% yield., m.p. 215°C. Requires: C, 57.83; H, 3.78; N, 8.63; S, 11.01; Cl, 12.22; Found: C, 57.9; H, 3.9; N, 9.7; S, 11.1; Cl, 12.3; IR (cm^{-1}): 3210, 3140, 2706, 1650, 1012, 620 (C-Cl).

10e as yellow crystals in 60% yield, m.p. 131°C. Requires: C, 69.39; H,5.06; N,10.1;S,7.71; Found: C, 69.4; H, 5.1; N, 10.2; S, 7.8; IR (cm^{-1}): 3258, 1650, 1026.

Synthesis of 2-amino-4,6-diaryl-2H-1,3-oxazine derivatives (11a-c).

A mixture of **1d,f,h**, (0.01 mol) and urea (0.01 mol) in 15 ml of absolute ethanol containing 6 ml of glacial acetic acid was heated under reflux for 6h. The precipitate that formed after cooling was collected, washed well with dilute ethanol and recrystallized from the proper solvent to give **11a-c**, respectively.

11a as Brown crystals in 60% yield, m.p. 194°C. Requires: C, 67.01; H, 5.23; N, 9.77;Cl, 12.39; Found C, 67.1; H, 5.3; N, 9.8;Cl, 12.4; IR (cm^{-1}): 3348, 1652, 1210, 516.

11b as white crystals in 70% yield, m.p. 109°C. Requires: C, 67.48; H, 4.56; N, 9.84; Cl, 12.47; Found: C, 67.5; H, 4.6; N, 9.9; Cl, 12.5; IR (cm^{-1}): 3200, 1654, 1212, 686. ^{1}H–NMR (DMSO-d_6): δ 2.1 (s, 2H, NH_2), 3.81, 3.82 (d, 1H, CHa), 6.9, 7.02 (d, 1H, CHb), 7.5-8.1 (m, 9H, Ar-H). ^{13}C-NMR: δ 37.2 (C_1), 161.1 (C_2), 97.07 (C_3), 168.8 (C_4), 130.5 (C_5), 127.7 (C_6), 139.3 (C_7), 143.4 (C_8), 134.5 (C_9), 127.3 (C_{10}), 143.1 (C_1`), 129.6 ($C_3$`), 131.8 (C_6`), 128.3 ($C_5$`), 127.7 (C_2`), 119.4 ($C_4$`).

11c as white crystals in 60% yield, m.p. 210°C. Requires: C, 72.18; H, 5.26; N, 10.52; Found: C, 72.2; H, 5.3; N, 10.6; IR (cm^{-1}): 3286, 1662, 1260.

Synthesis of nucleoside derivatives (13).

A suspension of **2** (0.01 mol) in 6 ml of aqueous potassium hydroxide (prepared by dissolving 0.01 mol in 6 ml of distilled water) was stirred by using a magnetic stirrer for 3h, then a solution of **12** (0.0 mol) dissolved in 30 ml of dry acetone was added drop-wise while stirring which continued for 12 h. After evaporation of the solvent (reduced pressure), the residue was washed with dilute ethanol several times and the precipitate formed was recrystallised from ethanol to give **13** as brown crystals in 60% yield, m.p. 168°C. Requires: C, 64.92; H, 5.41; N, 7.32; Found: C, 65.0; H, 5.4; N, 7.2. IR (cm^{-1}): 3346.2, 3000.9, 2840.3, 1663.6, 1600.1. ^{1}H–NMR (DMSO-d_6): δ 6.9, 7.0 (d, H-1' and H-2'), 2.41, 2.43 (s, 3H, 2x$COCH_3$), 10.04 - 10.52 (s, 1H, 2xOH).

Synthesis of (2R,3S,4R,5S)-5-hydroxy-2-[3-oxo-(4-phenyl-5-(2-chloro-phenyl)-3,3a,4,5-tetrahydro-2H-indazol-2-yl)tetrahydro-2H-pyran-3,4-diyl diacetate (14).

A suspension of **4** (0.01 mol) in 6ml of aqueous potassium hydroxide (prepared by dissolving 0.01 mol in 6ml of distilled water) was stirred using a magnetic stirrer for 3 h, then a solution of **12** (0.01 mol) dissolved in 30ml of dry acetone was added drop-wise while stirring, which continued for 12 h. After evaporation of the solvent (reduced pressure), the residue left was washed with dilute ethanol (several times) and the precipitate formed was re-crystallized from ethanol to give **14** as grey crystals, in 60% yield, m.p.102°C. Requires: C, 62.51; H, 4.83; N, 5.21; Cl, 6.60; Found: C, 62.5; H, 4.8; N, 5.3; Cl, 6.7; IR (cm^{-1}): 3443, 1742, 1606 and 1376.

Synthesis of nucleoside derivatives 15 and 16.

A suspension of the cyanopyridone derivatives **5c** and/or 2-imino-cyanopyridine **6c** (0.01 mol) in 6ml of aqueous KOH solution (prepared from dissolving (0.01 mol) solid KOH in 6ml of distilled water) was well stirred (magnetic stirrer) at room temperature for 3 h, then a solution of **12** (0.01 mol) dissolved in 30ml of dry acetone was added drop-wise while stirring. Stirring was continued for further 12 h. After evaporation of the excess solvent (reduced pressure), the residue left was washed with dilute alcohol (several times) and the precipitate formed was recrystallized from ethanol to give **15** and **16**.

15 as brown crystals, in 70% yield, m.p. 70°C. Requires: C, 56.55; H, 4.34; N, 5.28; Cl, 6.69; Found: C, 56.6; H, 4.5; N, 5.4; Cl, 7.1; IR (cm^{-1}): 3315.4, 1648, 1595, 525.8 (C-Cl). ^{1}H–NMR (DMSO-d_6): δ 4.41 (s, 1H, NH), 6.68-7.90 (m, 7H, Ar-H), 8.05 (d, 2H, 2xCH), 8.07(s, 1H, OH), 3.3, 3.41, 3.44 (t, 2H, CH_2), 8.07 (s, 1H, NH_2). MS (*m/z*, %): 497.45 (1.1%) M^+, 461 (1.05%), 232 (15.4%), 190 (1.09%), 148 (1.37%).

16 as black crystals in 60 % yield, m.p. 76°C. Requires: C, 56.65; H, 4.53; N, 7.93; Cl, 6.7; Found: C, 56.87; H, 4.67; N, 8.1; Cl, 6.9; IR (cm^{-1}): 3314 (br), 1655, 1584, 526 (C-Cl). ^{1}H-NMR (DMSO-d_6): δ 1.97, 2.49 (s, 3H, 2x$COCH_3$), 6.55 (s, 1H, NH), 2.44, 3.44 (d, 2H, 2xCH), 6.82-7.94 (m, 7H, Ar-H), 8.12 (s, 2H, NH_2) , 9.75 (s, 1H, OH). MS (*m/z*, 511.5 (0.4%), 484.5 (0.4%), 473.5 (0.69%), 426 (0.45%) and the base peak at m/z 50.

Synthesis of 2R, 3S, 4R, 5S-2-(4,6-diaryl-3-carbonitrile) -2-thioxo-1(2H)-pyridin-yl)-5'-hydroxy-tetrahydro-2H-pyran-3,4-di-yl) diacetate (17a,b).

A suspension of the thiocyanopyridine derivatives **7a** and/or **7b** (0.01 mol) in 6ml of aqueous KOH solution (prepared from dissolving (0.01 mol) solid KOH in 6ml of distilled water) was well stirred (magnetic stirrer) at room temperature for 3 hrs, then a solution of **12** (0.01 mol) dissolved in 30ml of dry acetone was added drop-wise while stirring. Stirring was continued for further 12 hrs. After evaporation of the excess solvent (reduced pressure), the residue left was washed with dilute alcohol (several times) and the precipitate formed was recrystallized from ethanol as **17a,b**.

17a as red crystals, in yield 70% ,m.p 146°C.[Requires: C, 59.10; H, 4.39; N, 4.92; Cl, 6.24;S,5.62 ; Found C, 59.32; H, 4.5; N,5.10 ; Cl, 6.34;S,5.78 %]; IR cm^{-1}3436.5 ,2220.4, 1600.6,1248.6 and 526.4 for OH, C≡N,C=N,C=S and C-Cl.

17b as black crystals, in 60% yield, m.p. 184°C. Requires: C, 56.76; H, 3.97; N, 5.29; Cl, 6.72; S, 6.05; Found: C, 56.87; H, 4.01; N, 5.43; Cl, 6.9; S, 6.32; IR (cm^{-1}): 3300, 2228, 1597, 1232 and 525 (C-Cl).

Synthesis of 2S,3S,4R,5S-2-(6-(4-chlorophenyl)-4-(2-bromophenyl)-2,3,4,5-tetrahydro-pyrimidin-1-yl)mercapto-5-hydroxy tetrahydro-2H-pyran-3,4-diyl diacetate (18a) and 2S,3S,4R,5S-2-(6-(phenyl)-4-(2-bromophenyl)-2,3,4,5-tetra-hydro-pyrimidin-1-yl)-mercapto-5-hydroxy tetrahydro-2H-pyran-3,4-diyl diacetate (18b).

A suspension of the pyrimidin-2-thione derivatives **(10a)** and/or **(10c)** (0.01 mol) in 6ml of aqueous KOH solution (prepared from dissolving (0.01 mol) solid KOH in 6ml of distilled water) was well stirred (magnetic stirrer) at room temperature for 3 h, then a solution of **12** (0.01 mol) dissolved in 30ml of dry acetone was added drop-wise while stirring. Stirring was continued for further 12 h. After evaporation of the excess solvent (reduced pressure), the residue left was washed with dilute alcohol (several times) and the precipitate formed was recrystallized from ethanol to give (**18a,b)**.

18a as grey crystals , in 70% yield, m.p. 188°C. Requires: C, 50.46; H, 3.86; N, 4.71; S; 5.38; Cl, 6.24; Br, 13.4; Found: C, 50.52; H, 3.98; N, 4.81; S, 5.45; Cl, 5.45; Br, 13.5; IR (cm^{-1})3398, 2652,1671, 1563, 625 and 518 (C-Br). ^{1}H-NMR spectrum (DMSO-d_6): δ 1.19, 2.49 (s, 6H, 2x $COCH_3$), δ 3.32(d, 2H, CH_2), 4.29 (t, 1H, CH), 5.37, 5.38(d, 1H, CH), 5.40, 5.41 (d, 1H, CH), 7.23-7.64 (m, 9H, Ar–H), 9.05 (s, 1H, SH), 9.99 (s, 1H, OH).

18b as white crystals, in 75% yield, m.p. 154°C. Requires: C, 53.57; H, 4.28; N, 5.00; S, 5.71; Br, 14.3; Found: C, 53.87; H, 4.31; N, 5.01; S, 5.82; Br, 14.4; IR (cm^{-1}): 3394, 2644, 1657, 1569, 549 (C-Br). 1H-NMR (DMSO-d6): δ 1.01, 2.49 (s, 6H, 2x $COCH_3$), 3.43 (d, 2H, CH_2), δ 4.29 (d, 2H, CH_2), 5.39 (t, 1H, CH), 7.06-7.96 (m, 8H, Ar–H), 9.09 (s, 1H, SH), 10.10 (s, 1H, OH). MS (*m/z*, %): 424 M^{+2} (0.02%).

Synthesis of nucleoside derivatives (19a-c).

A suspension of pyrimidin-2-thione derivatives (**10b,d,e)** (0.01 mol) in 6ml of aqueous KOH solution (prepared from dissolving (0.01 mol) solid KOH in 6ml of distilled water) was well stirred (magnetic stirrer) at room temperature for 3 h, then a solution of **12** (0.01 mol) dissolved in 30ml of dry acetone was added drop-wise while stirring. Stirring was continued for further 12 h. After evaporation of the excess solvent (reduced pressure), the residue left was washed with dilute alcohol (several times) and the precipitate formed was recrystallized from ethanol to give (**19a-c)**.

19a, as Grey crystals, in 70% yield, m.p. 120°C. Requires: C, 54.64; H, 4.01; N, 5.10; S, 5.82; Cl; 12.9; Found: C, 50.72; H, 4.21; N, 5.21; S, 5.95; Br, 13.1; IR (cm^{-1}): 3294, 1674, 1552,1256, 728 (C-Cl). ^{1}H-NMR (DMSO-d_6): δ 2.49, 2.50 (d, 2H, CH_2), 3.33, 3.44, 3.59 (t, 1H-CH), 3.71, 4.33 (d, 1H, CH1'–CH2'), 5.380, 5.385 (d, 1H, CH2'), 5.39, 5.40 (d,1H, CH3'), 5.44, 5.45, 5.46 (t, 1H, CH4'), 5.47, 6.65 (d, 2H, $CH_2$5'), 6.82-7.73 (m, 8H, Ar–H), and at 10.06 (s, 1H, OH). MS (*m/z*, %): 478 (0.55%).

19b as orange crystals, in 75% yield, m.p. 190°C. IR (cm^{-1}): 3401, 1670, 1595, 1240, 752 (C-Cl). ^{1}H-NMR (DMSO-d_6): δ 2.49, 2.50 (d, 2H, CH_2), 3.43 (t, 1H, CH), 6.76, 6.77 (d, 1H, $CH_2$5'), 6.99-7.61 (m, 7H, Ar–H), 8.18(s, 1H, OH). MS (*m/z*, %): 480 M^{+1} (0.22%).

19c as black crystals, 60% yield, m.p. 224°C. Requires: C, 54.71; H, 4.16; N, 5.55; S, 6.34; Cl, 7.03; Found C, 54.89; H, 4.32; N, 5.76; S, 6.56; Cl, 7.12; IR (cm^{-1}): 3343-3225, 1740, 1602, 1243. ^{1}H-NMR (DMSO-d_6): δ 3.55 (s, 3H, OCH_3), 5.93 (s, 1H, NHCO), 7.01-7.90 (m, 13H, Ar–H), 10.1 (s, 1H, OH). MS (*m/z*, %): 300 M^{+2} (0.59%).

Synthesis of (2R,3S,4R,5S)-2(4(-2-chlorophenyl)-6-phenyl-4'-1,3-oxazine-2-ylamino)-5'-hydroxytetrahydro -2H-pyran-3',4'-diyl diacetate (20a) and (2R,3S,4R,5S)-2(6-(4-benzamidophenyl)-4-(4-methoxyphenyl)-4H-1,3-oxazin-2-ylamino)-5'-hydroxytetrahydro-2H-pyran-3',4'-diyl diacetate (20b).

A suspension of the Oxazine derivatives **11a** and/or **11b** (0.01 mol) in 6ml of aqueous KOH solution (prepared from dissolving (0.01 mol) solid KOH in 6ml of distilled water) was well stirred (magnetic stirrer) at room temperature for 3 h, then a solution of **12** (0.01 mol) dissolved in 30ml of dry acetone was added drop-wise while stirring. Stirring was continued for further 12 h. After evaporation of the excess solvent (reduced pressure), the residue left was washed with dilute alcohol (several times) and the precipitate formed was recrystallized from ethanol to give **20a,b**.

20a as pale yellow crystals, 60% yield, m.p. 158-160°C. Requires: C, 64.39; H, 5.36; N, 6.82; Found: C, 64.94; H, 5.40; N, 6.8. IR (cm^{-1}): 3482, 3302, 1654, 1666, 1591. ^{1}H-NMR (DMSO-d_6): δ 2.49, 2.495 (s, 6H, 2x$COCH_3$), 3.91 (s, 2H, CH_2), 4.0 (d, 2H, CH – CH), 4.06 (s, 1H, NH), 7.42 – 7.82 (m, 9H, Ar-H), 8.05 (s, 1H, OH).

20b as yellow crystals, 60% yield, m.p. 110-112°C. Requires: C, 59.94; H, 4.99; N, 5.59; Cl, 7.09; Found: C, 60.1; H, 5.0; N, 5.6; Cl, 7.1. IR (cm^{-1}): 3482, 3302, 1654, 1666, 1591, 1593. ^{1}H-NMR (DMSO-d_6): δ 6.9 d, 2H-CH-CH) 2.1 - 2.6 (s, 6H, 2x$COCH_3$), 10.2 (s, 1H, NH), 10.5 (s, 1H, OH), 3.8 (s, 3H, OCH_3), and 7.02 – 8.13 (m, 13H-Ar). ^{13}C-NMR (DMSO-d_6): δ 24.1, 26.4, 38.6, 55.3.

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