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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, 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

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).

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, 13C 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 13C. 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) (0.01 mol) and hydrazine hydrate (0.01 mol) in 30 ml of ethanol was refluxed for 6 h. 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) (0.01 mol) and ethylacetoacetate (0.01 mol) in 30 ml of absolute ethanol containing sodium ethoxide (prepared from 0.2 g of sodium metal and 4.6 ml of absolute ethanol) was refluxed for 6 h. 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 6 h. 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, 11.1 IR (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%).

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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 6 h. 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 3 h. 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 (0.01 mol) and P2S5 (0.01 mol) in 15 ml of dry xylene was refluxed for 6 h. 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) (1 g) of and (5 ml) of 30-40% H_2SO_4 in 15 ml acetic acid was refluxed for 6 h. 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 3 h. 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 (0.01 mol) in 30 ml ethanol containing (0.01 mol) sodium ethoxide was heated under reflux for 6 h. 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 6 h. 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-d6): δ 2.1 (s, 2H, NH2), 3.81, 3.82 (d, 1H, CHa), 6.9, 7.02 (d, 1H, CHb), 7.5-8.1 (m, 9H, Ar-H). 13C-NMR: δ 37.2 (C1), 161.1 (C2), 97.07 (C3), 168.8 (C4),

130.5 (C5), 127.7 (C6), 139.3 (C7), 143.4 (C8), 134.5 (C9), 127.3 (C10), 143.1 (C1`), 129.6 (C3`), 131.8 (C6`), 128.3 (C5`), 127.7 (C2`), 119.4 (C4`).

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 3 h, 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. 1H–NMR (DMSO-d6): 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 6 ml of aqueous potassium hydroxide (prepared by dissolving 0.01 mol in 6 ml of distilled water) was stirred using a magnetic stirrer for 3 h, then a solution of 12 (0.01 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 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 6 ml of aqueous KOH solution (prepared from dissolving (0.01 mol) solid KOH in 6 ml of distilled water) was well stirred (magnetic stirrer) at room temperature for 3 h, then a solution of 12 (0.01 mol) dissolved in 30 ml 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 6 ml of aqueous KOH solution (prepared from dissolving (0.01 mol) solid KOH in 6 ml of distilled water) was well stirred (magnetic stirrer) at room temperature for 3 hrs, then a solution of 12 (0.01 mol) dissolved in 30 ml 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 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-13436.5, 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 6 ml of aqueous KOH solution (prepared from dissolving (0.01 mol) solid KOH in 6 ml of distilled water) was well stirred (magnetic stirrer) at room temperature for 3 h, then a solution of 12 (0.01 mol) dissolved in 30 ml 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). ^{1}H-NMR (DMSO-d_6): 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 6 ml of aqueous KOH solution (prepared from dissolving (0.01 mol) solid KOH in 6 ml of distilled water) was well stirred (magnetic stirrer) at room temperature for 3 h, then a solution of 12 (0.01 mol) dissolved in 30 ml 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-d6): 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, CH25'), 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-d6): δ 2.49, 2.50 (d, 2H, CH_2), 3.43 (t, 1H, CH), 6.76, 6.77 (d, 1H, CH25'), 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 6 ml of aqueous KOH solution (prepared from dissolving (0.01 mol) solid KOH in 6 ml of distilled water) was well stirred (magnetic stirrer) at room temperature for 3 h, then a solution of 12 (0.01 mol) dissolved in 30 ml 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.

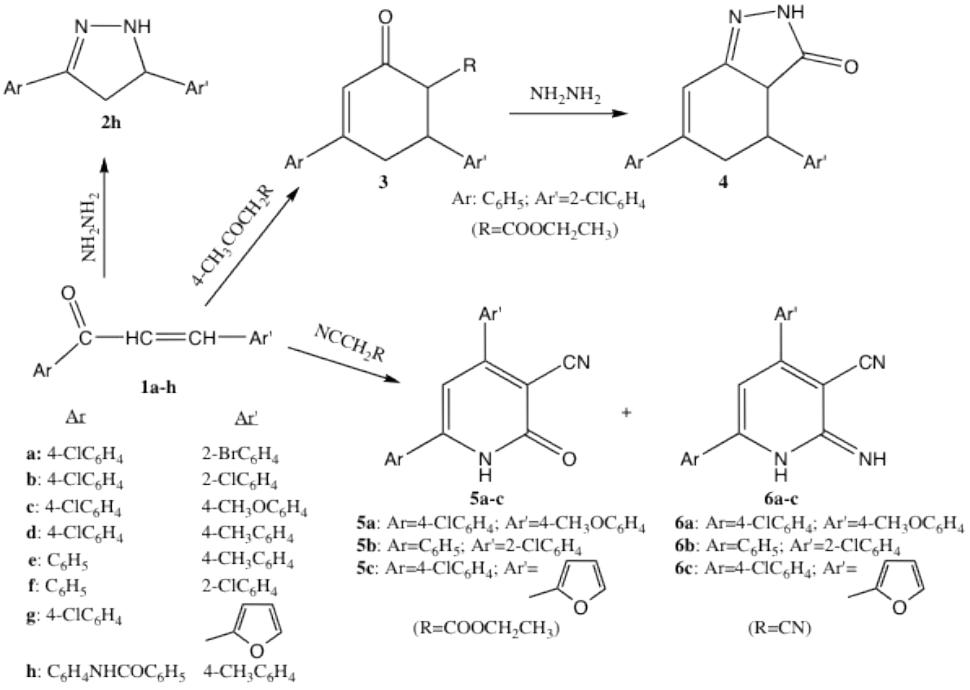
20a as pale yellow crystals, 60% yield, m.p. 158-160°C. Requires: C, 64.39; H, 5.36; N, 6.82; Found: C, 64.94; H, 5.40; N, 6.8. IR (cm^{-1}): 3482, 3302, 1654, 1666, 1591. ^{1}H-NMR (DMSO-d_6): δ 2.49, 2.495 (s, 6H, 2x$COCH_3$), 3.91 (s, 2H, CH_2), 4.0 (d, 2H, CH – CH), 4.06 (s, 1H, NH), 7.42 – 7.82 (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, ^{13}H-Ar). ^{13}C-NMR (DMSO-d_6): δ 24.1, 26.4, 38.6, 55.3.

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.



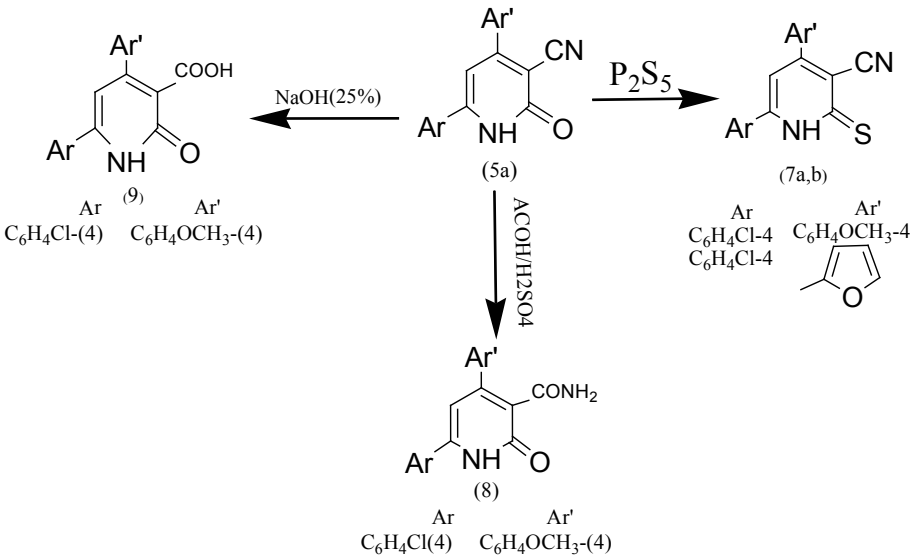
Scheme 1: Heterocycle synthesis from 1,3-diaryl-1-propen-3-ones.

Reaction of 1c, f, g with active methylene compounds 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 (Scheme 1). 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.

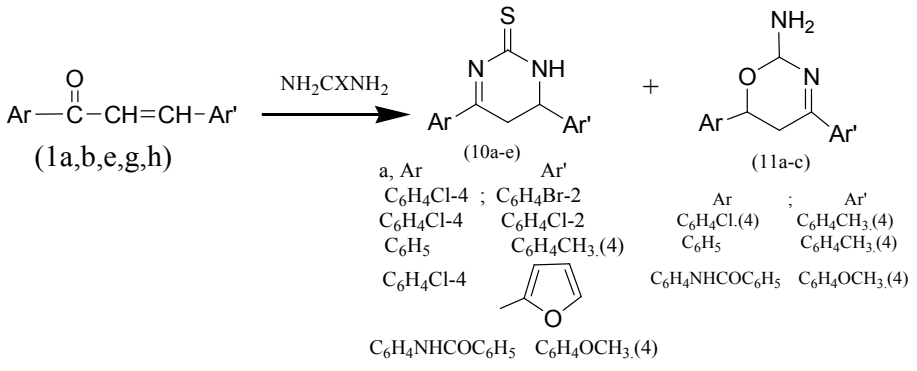
Scheme 2 depicts the reactions undertaken with cyanopyridone derivatives 5a,c. The reaction of 5a,c with phosphorus pentasulphide in a non-polar solvent, (e.g., xylene under reflux) afforded the corresponding 4,6-diaryl-2-thioxo-1,2-dihydropyridin-3-carbonitrile derivatives 7a-b. The IR spectra showed 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 CN.

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



Scheme 2: Functionalizations of 5a,c.



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

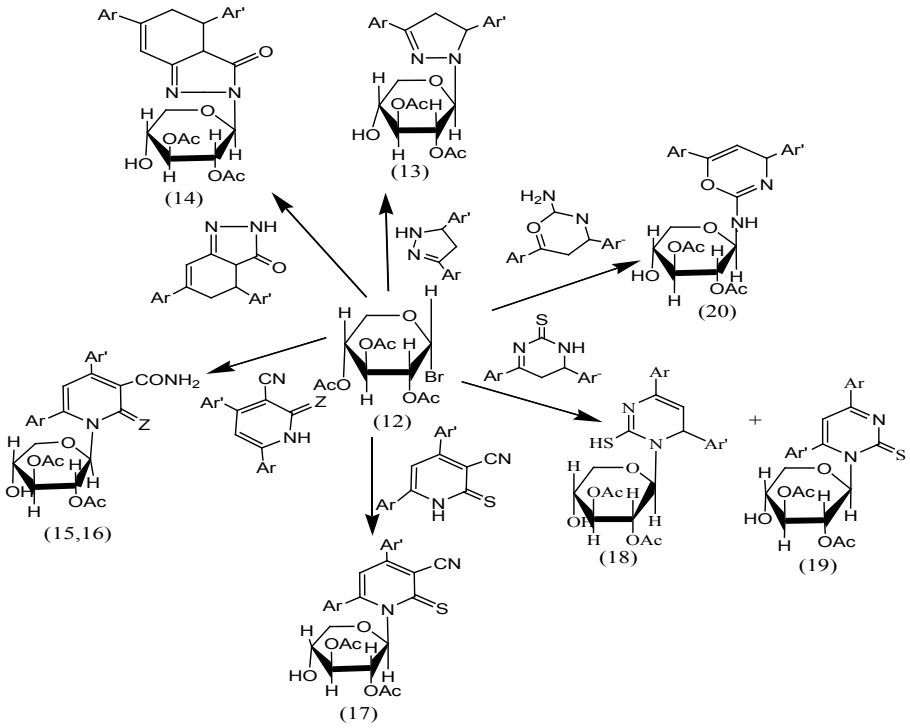
and a multiplet for the aromatic protons at δ 7.5-8.1 ppm. The ^{13}C-NMR spectrum showed signals at δ 37.2(C_1), 161.1 (C_2), 97.07(C_3), 168.8 (C_4), 130.5 (C_5), 127.7 (C_6), 139.3 (C_7), 143.4 (C_8), 134.5 (C_9), 127.3 (C_{10}), 143.1 (C_1`), 129.6 ($C_3$`), 131.8 (C_6`), 128.3 ($C_5$`), 127.7 (C_2`), 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 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 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 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



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

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-2H-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.

Biological Evaluation

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 5×10^5 c.f.u./ml of actively dividing bacteria cells. The cultures were incubated for 24 h. 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 h 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.

Antifungal studies

Antifungal activity testing was also done by the disk diffusion method [43]. For assaying antifungal activity *Aspergillus fumigatus*

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	--	--	--	--

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

(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.

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 (Tables 3 and 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.

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

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.

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

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 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]			
	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, carcinoma cells HepG2, Breast carcinoma cells MCF-7; [b]Assays were performed in triplicate. NT indicates not tested.

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

[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.

Conclusion

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 epidermis/larynx cancer cell line. Compounds 4, 5c, 10c, 11c, 14, 17a, 18a and 20b exhibited good antitumor activity when compared with the reference drug.

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