# Supplementary Material

## Design and Synthesis of Pyridine-Pyrazole-Sulfonate Derivatives as Potential Anti-HBV Agents

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**Experimental Section:**

## 1. Chemistry

All reactions were carried out in oven-dried glassware (120 °C) under an atmosphere of nitrogen. Ethyl acetate and hexane from Mallinckrodt Chemical Co. were dried and distilled from $CaH_2$. Tetrahydrofuran was dried and distilled from sodium. 2-Acetylpyridine, benzenesulfonyl chloride, 4-bromobenzenesulfonyl chloride, 4-chlorobenzenesulfonyl chloride, diethyl carbonate, ethyl 4-chloroacetoacetate, 4-fluorobenzenesulfonyl chloride, hydrazine hydrate, 4-methoxybenzenesulfonyl chloride, 2-mercaptopyridine, 4-nitrobenzenesulfonyl chloride, peaonol (2'-hydroxy-4-methoxyacetophenone), potassium carbonate, sodium hydride and *p*-toluenesulfonyl chloride were purchased from Sigma-Aldrich Chemical Co without further purification.

Analytical thin layer chromatography (TLC) was performed on precoated plates (silica gel 60 F–254), purchased from Merck Inc. Purification by gravity column chromatography was carried out by use of Merck Reagents Silica Gel 60 (particle size 0.063–0.200 □mm, 70-230 mesh ASTM). Infrared (IR) spectra were measured on a Bruker ALPHA-P FTIR spectrometer. Absorption intensities are recorded by the following abbreviations: s, strong; m, medium; w, weak. Proton NMR spectra were obtained on a Bruker Avance 500 (500 MHz) and Mercury 400 (400 MHz) by use of chloroform-*d* and dimethyl sulfoxide-$d_6$ as solvent. Proton NMR chemical shifts are referenced to the $CHCl_3$ singlet (7.24 ppm) and DMSO pentet (2.49 ppm). Carbon-13 NMR spectra were obtained on a Bruker Avance 500 (125 MHz) and Mercury 400 (100 MHz) by use of chloroform-*d* and dimethyl sulfoxide-$d_6$ as solvent. Carbon-13 chemical shifts are referenced to the center of the $CDCl_3$ triplet (77.0 ppm) and DMSO septet (39.5 ppm). Multiplicities are recorded by the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; *J*, coupling

constant (hertz). High-resolution mass spectra were obtained by means of a FINNIGAN /MAT-95XL mass spectrometer. High-performance liquid chromatography (HPLC) analyses were carried out by Agilent 1100 series system with CNW Athena C18 column (120 Å, 4.6 mm × 250 mm, 5 μm) and UV detection at 254 nm. Ethyl 4-(pyridin-2-ylthio)acetoacetate **12** and ethyl picolinoylacetate **17** were synthesized in accordance with literature procedure.[1, 2]

**1.1 1,2-Dihydro-5-[(2-pyridinylthio)methyl]-3*H*-pyrazol-3-one. (13)**

To a reaction vessel containing ethyl 4-(pyridin-2-ylthio)acetoacetate 12 (1.0 g, 1.0 equiv.) in 5.0 mL tetrahydrafuran and 5.0 mL ethanol was added hydrazine hydrate (50%) (0.3 mL, 2.5 equiv.) and stirred at room temperature for 2.0 hours. It was quenched with water and solvent was removed under reduced pressure. The residue was extracted with ethyl acetate (3 × 30 mL). The combined organic layer were washed with brine, dried over $MgSO_{4(S)}$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (ethyl acetate and hexane as eluent) to give desired products (0.75 g, 86% yield). $^1$H NMR (DMSO-$d_6$, 400 MHz) $\delta$ 8.45 (d, $J$ = 4.4 Hz, 1 H, H-6), 7.64 (t, $J$ = 8.0 Hz, 1 H, H-4), 7.30 (d, $J$ = 8.0 Hz, 1 H, H-3), 7.12 (t, $J$ = 6.4 Hz, 1 H, H-5), 5.34 (s, 1 H, H-4′), 4.24 (s, 2 H, $SCH_2$).

**1.2 Standard procedure for the conjugation of pyridine-$SCH_2$-pyrazole with benzenesulfonyl chloride derivatives. (14a to 14h)**

To a reaction vessel containing 1,2-dihydro-5-[(2-pyridinylthio)methyl] -3H-Pyrazol-3-one **13** (1.0 equiv.), benzenesulfonyl chloride derivatives (1.2 equiv.) and potassium carbonate (2.0 equiv.) in 10.0 mL tetrahydrofuran was stirred at 40 °C for 4.0–5.0 hours. It was quenched with water and solvent was removed under reduced pressure. The residue was extracted with ethyl acetate (3 × 30 mL). The combined organic layer were washed with brine, dried over $MgSO_{4(S)}$, filtered, and

concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (ethyl acetate and hexane as eluent) to give desired products. All the products with purity of >95.0% were checked by HPLC.

**1.3 1,2-Dihydro-5-(2-pyridinyl)-3*H*-Pyrazol-3-one. (18)**

To a reaction vessel containing ethyl picolinoylacetate **17** (1.0 g, 1.0 equiv.) in 10.0 mL ethanol was added hydrazine hydrate (0.4 mL, 1.2 equiv.) and stirred at room temperature for 2.0 hours. It was quenched with water and solvent was removed under reduced pressure. The residue was extracted with ethyl acetate (3 × 30 mL). The combined organic layer were washed with brine, dried over $MgSO_{4(S)}$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (ethyl acetate and hexane as eluent) to give desired products (0.66 g, 79% yield). $^{1}$H NMR (*d*-Acetone, 400 MHz) $\delta$ 8.53 (d, $J$ = 4.4 Hz, 1 H, H-6), 7.80 (t, $J$ = 7.6 Hz, 1 H, H-4), 7.73 (d, $J$ = 8.0 Hz, 1 H, H-3), 7.27 (t, $J$ = 6.0 Hz, 1 H, H-5), 6.03 (s, 1 H, H-4′); $^{13}$C NMR (DMSO, 100 MHz) $\delta$ 160.98, 149.28, 137.08, 122.72, 119.49, 88.03.

**1.4 Standard procedure for the conjugation of pyridine-pyrazole with benzenesulfonyl chloride derivatives. (19a to 19h)**

To a reaction vessel containing 1,2-Dihydro-5-(2-pyridinyl)-3H-Pyrazol-3-one **18** (1.00 equiv.), benzenesulfonyl chloride derivatives (1.20 equiv.) and potassium carbonate (2.00 equiv.) in 10.0 mL tetrahydronfuran was stirred at 40 °C for 4.0–5.0 hours. It was quenched with water and solvent was removed under reduced pressure. The residue was extracted with ethyl acetate (3 × 30 mL). The combined organic layer were washed with brine, dried over $MgSO_{4(S)}$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (ethyl acetate and hexane as eluent) to give desired products. All the products with purity of >95.0% were checked by HPLC.

**1.5 5-[(Pyridin-2-ylthio)methyl]-1*H*-pyrazol-3-yl benzenesulfonate 14a**: 77% yield, mp (recrystallized from $CH_2Cl_2$) 233.5–234.5 °C; $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 8.40–8.39 (m, 1 H, H-6), 7.89–7.87 (m, 2 H, 2 × ArH), 7.62–7.58 (m, 1 H, ArH), 7.51–7.45 (m, 3 H, H-4 + 2 × ArH), 7.18 (d, $J$ = 8.0 Hz, 1 H, H-3), 7.04–7.01 (m, 1 H, H-5), 5.92 (d, $J$ = 8.4 Hz, 2 H, 2 × ArH), 5.90 (s, 1 H, H-4′), 4.15 (s, 2 H, $SCH_2$); $^{13}$C NMR ($CDCl_3$, 100 MHz) $\delta$ 158.04, 153.51, 149.33, 142.94, 136.62, 135.35, 134.17, 128.98, 128.38, 122.77, 120.27, 95.25, 24.50 ($SCH_2$); IR (neat) 3711 (m), 1619 (m), 1543 (s), 1372 (s), 1176 (m), 1109 (m), 960 (m) cm$^{-1}$; ESIMS calcd for ($C_{15}H_{13}N_3O_3S_2$): 347.0398; found 347.0398.

**1.6 5-[(Pyridin-2-ylthio)methyl]-1*H*-pyrazol-3-yl 4-methylbenzenesulfonate 14b**: 85% yield, mp (recrystallized from $CH_2Cl_2$) 231.5–232.5 °C; $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 8.37 (d, $J$ = 4.8 Hz, 1 H, H-6), 7.72 (d, $J$ = 8.4 Hz, 2 H, 2 × ArH), 7.48–7.44 (m, 1 H, H-4), 7.23 (d, $J$ = 8.4 Hz, 2 H, 2 × ArH), 7.14 (d, $J$ = 8.0 Hz, 1 H, H-3), 7.01–6.98 (m, 1 H, H-5), 5.87 (s, 1 H, H-4′), 4.12 (s, 2 H, $SCH_2$), 2.35 (s, 3 H, $CH_3$); $^{13}$C NMR ($CDCl_3$, 100 MHz) $\delta$ 157.88, 153.48, 149.24, 145.29, 145.27, 142.80, 136.53, 132.12, 129.55, 128.40, 128.28, 122.64, 120.17, 95.18, 24.38 ($SCH_2$), 21.52 ($CH_3$); IR (neat) 3700 (m), 1620 (m), 1349 (s), 1264 (s), 1113 (m), 1042 (m) cm$^{-1}$; ESIMS calcd for ($C_{16}H_{15}N_3O_3S_2$): 361.0555; found 361.0554.

**1.7 5-[(Pyridin-2-ylthio)methyl]-1*H*-pyrazol-3-yl 4-nitrobenzenesulfonate 14c**: 76% yield, mp (recrystallized from $CH_2Cl_2$) 230.5–231.5 °C; $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 8.42–8.40 (m, 1 H, H-6), 8.30 (d, $J$ = 5.6 Hz, 2 H, 2 × ArH), 8.09 (d, $J$ = 6.4 Hz, 2 H, 2 × ArH), 7.54–7.49 (m, 1 H, H-4), 7.20 (d, $J$ = 8.0 Hz, 1 H, H-3), 7.07–7.04 (m, 1 H, H-5), 5.90 (s, 1 H, H-4′), 4.15 (s, 2 H, $SCH_2$); $^{13}$C NMR ($CDCl_3$, 100 MHz) $\delta$ 157.98, 153.23, 150.82, 149.35, 143.46, 141.02, 136.75, 129.90, 124.34, 124.13, 122.88, 120.40, 95.34, 24.52 ($SCH_2$); IR (neat) 3732 (m), 1643 (m), 1572 (s), 1376 (s), 1189 (m), 1106 (m), 990 (m) cm$^{-1}$; ESIMS calcd for ($C_{15}H_{12}N_4O_5S_2$):

392.0249; found 392.0248.

**1.8 5-[(Pyridin-2-ylthio)methyl]-1*H*-pyrazol-3-yl 2-nitrobenzenesulfonate**

**14d**: 79% yield, mp (recrystallized from $CH_2Cl_2$) 229.7–230.5 °C; $^1$H NMR (*d*-Acetone, 400 MHz) $\delta$ 8.44 (d, $J$ = 4.8 Hz, 1 H, H-6), 8.05 (d, $J$ = 7.6 Hz, 1 H, ArH), 8.00–7.93 (m, 2 H, 2 × ArH), 7.83–7.78 (m, 1 H, ArH), 7.59–7.54 (m, 1 H, H-4), 7.20 (d, $J$ = 8.0 Hz, 1 H, H-3), 7.08–7.05 (m, 1 H, H-5), 6.01 (s, 1 H, H-4′), 4.38 (s, 2 H, $SCH_2$); $^{13}$C NMR ($CDCl_3$, 100 MHz) $\delta$ 157.92, 154.17, 150.34, 149.22, 143.74, 137.41, 136.86, 133.25, 132.56, 128.90, 125.78, 122.84, 120.86, 96.08, 24.31 ($SCH_2$); IR (neat) 3732 (m), 1643 (m), 1572 (s), 1376 (s), 1189 (m), 1106 (m), 990 (m) cm$^{-1}$; ESIMS calcd for ($C_{15}H_{12}N_4O_5S_2$): 392.0249; found 392.0248.

**1.9 5-[(Pyridin-2-ylthio)methyl]-1*H*-pyrazol-3-yl 4-fluorobenzenesulfonate**

**14e**: 81% yield, mp (recrystallized from $CH_2Cl_2$) 233.5–234.5 °C; $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 8.41–8.39 (m, 1 H, H-6), 7.91–7.88 (m, 2 H, 2 × ArH), 7.51–7.47 (m, 1 H, H-4), 7.19-7.11 (m, 3 H, H-3 + 2 × ArH), 7.05–7.01 (m, 1 H, H-5), 5.92 (s, 1 H, H-4′), 4.17 (s, 2 H, $SCH_2$); $^{13}$C NMR ($CDCl_3$, 100 MHz) $\delta$ 167.10, 164.55, 157.74, 153.29, 149.26, 149.10, 142.97, 142.91, 136.55, 131.46, 131.33, 131.23, 131.16, 131.09, 131.06, 122.66, 120.20, 116.42, 116.36, 116.20, 116.14, 95.26, 24.36 ($SCH_2$); IR (neat) 3714 (m), 1618 (m), 1517 (s), 1399 (s), 1226 (s), 1061 (m) cm$^{-1}$; ESIMS calcd for ($C_{15}H_{12}N_3O_3FS_2$): 365.0304; found 365.0303.

**1.10 5-[(Pyridin-2-ylthio)methyl]-1*H*-pyrazol-3-yl 4-bromobenzenesulfonate**

**14f**: 83% yield, mp (recrystallized from $CH_2Cl_2$) 231.6–232.5 °C; $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 8.42 (d, $J$ = 4.8 Hz, 1 H, H-6), 7.74 (d, $J$ = 8.4 Hz, 2 H, 2 × ArH), 7.61 (d, $J$ = 8.4 Hz, 2 H, 2 × ArH), 7.51 (t, $J$ = 7.6 Hz, 1 H, H-4), 7.20 (d, $J$ = 8.4 Hz, 1 H, H-3), 7.06–7.03 (m, 1 H, H-5), 5.93 (s, 1 H, H-4′), 4.15 (s, 2 H, $SCH_2$); $^{13}$C NMR ($CDCl_3$, 100 MHz) $\delta$ 158.06, 153.41, 149.37, 143.16, 136.69, 134.35, 132.35, 129.91, 129.58, 122.86, 120.35, 95.33, 24.54 ($SCH_2$); IR (neat) 3691 (m), 1679 (m), 1559 (s),

1341 (s), 1232 (s), 1190 (m), 969 (m) $cm^{-1}$; ESIMS calcd for ($C_{15}H_{12}N_3O_3S_2Br$): 424.9503; found 424.9502.

**1.11 5-[(Pyridin-2-ylthio)methyl]-1*H*-pyrazol-3-yl 4-methoxybenzenesulfonate 14g**: 87% yield, mp (recrystallized from $CH_2Cl_2$) 235.1–236.3 °C; $^1H$ NMR ($CDCl_3$, 400 MHz) $\delta$ 8.41 (d, $J$ = 4.0 Hz, 1 H, H-6), 7.80 (d, $J$ = 8.8 Hz, 2 H, 2 × ArH), 7.52–7.48 (m, 1 H, H-4), 7.19 (d, $J$ = 8.0 Hz, 1 H, H-3), 7.05–7.02 (m, 1 H, H-5), 6.92 (d, $J$ = 8.4 Hz, 2 H, 2 × ArH), 5.90 (s, 1 H, H-4′), 4.15 (s, 2 H, $SCH_2$), 3.82 (s, 3 H, $OCH_3$); $^{13}C$ NMR ($CDCl_3$, 100 MHz) $\delta$ 164.05, 158.15, 153.71, 149.37, 142.89, 136.65, 130.71, 126.60, 122.82, 120.29, 114.22, 95.35, 55.60 ($OCH_3$), 24.57 ($SCH_2$); IR (neat) 3722 (m), 1668 (m), 1513 (s), 1364 (s), 1190 (m), 1146 (m), 981 (m) $cm^{-1}$; ESIMS calcd for ($C_{16}H_{15}N_3O_4S_2$): 377.0504; found 377.0503.

**1.12 5-[(Pyridin-2-ylthio)methyl]-1*H*-pyrazol-3-yl methanesulfonate 14h**: 76% yield, mp (recrystallized from $CH_2Cl_2$) 213.5–214.5 °C; $^1H$ NMR ($CDCl_3$, 400 MHz) $\delta$ 8.47–8.45 (m, 1 H, H-6), 7.54–7.50 (m, 1 H, H-4), 7.21 (d, $J$ = 8.0 Hz, 1 H, H-3), 7.08–7.05 (m, 1 H, H-5), 6.01 (s, 1 H, H-4′), 4.20 (s, 2 H, $SCH_2$), 3.23 (s, 3 H, $CH_3$); $^{13}C$ NMR ($CDCl_3$, 100 MHz) $\delta$ 158.09, 153.93, 149.36, 143.43, 136.73, 122.90, 120.39, 95.39, 37.98 ($CH_3$), 24.56 ($SCH_2$); IR (neat) 3689 (m), 1654 (m), 1391 (s), 1221 (s), 1121 (m), 1058 (m) $cm^{-1}$; ESIMS calcd for ($C_{10}H_{11}N_3O_3S_2$): 285.0242; found 285.0241.

**1.14 5-(Pyridin-2-yl)-1*H*-pyrazol-3-yl benzenesulfonate 19a**: 75% yield, mp (recrystallized from $CH_2Cl_2$) 225.3–226.5 °C; $^1H$ NMR (*d*-Acetone, 400 MHz) $\delta$ 8.58 (d, $J$ = 4.4 Hz, 1 H, H-6), 7.99–7.97 (m, 2 H, 2 × ArH), 7.85 (d, $J$ = 8.0 Hz, 1 H, H-3), 7.81–7.76 (m, 2 H, H-4 + ArH), 7.68-7.64 (m, 2 H, 2 × ArH), 7.36–7.33 (m, 1 H, H-5), 6.66 (s, 1 H, H-4′); $^{13}C$ NMR (*d*-Acetone, 100 MHz) $\delta$ 154.80, 149.43, 146.72, 145.54, 142.38, 137.35, 132.21, 129.77, 128.62, 123.65, 120.26, 94.51; IR (neat) 3721 (m), 1532 (s), 1484 (s), 1399 (s), 1202 (s), 1153 (m), 1095 (m), 953 (m) $cm^{-1}$;

ESIMS calcd for ($C_{14}H_{11}N_3O_3S$): 301.0521; found 301.0524.

**1.15 5-(Pyridin-2-yl)-1*H*-pyrazol-3-yl 4-methylbenzenesulfonate 19b**: 88% yield, mp (recrystallized from $CH_2Cl_2$) 227.5–228.5 °C; $^1$H NMR (*d*-Acetone, 400 MHz) $\delta$ 8.57–8.55 (m, 1 H, H-6), 7.80 (d, $J$ = 8.0 Hz, 2 H, 2 × ArH), 7.77–7.73 (m, 1 H, H-4), 7.55–7.53 (m, 1 H, H-3), 7.29 (d, $J$ = 8.0 Hz, 2 H, 2 × ArH), 7.26–7.23 (m, 1 H, H-5), 6.51 (s, 1 H, H-4′), 2.40 (s, 3 H, $CH_3$); $^{13}$C NMR (*d*-Acetone, 100 MHz) $\delta$ 154.80, 149.43, 146.72, 145.54, 142.38, 137.35, 132.21, 129.77, 128.62, 123.65, 120.26, 94.51, 21.72 ($CH_3$); IR (neat) 3721 (m), 1532 (s), 1484 (s), 1399 (s), 1202 (s), 1153 (m), 1095 (m), 953 (m) cm$^{-1}$; ESIMS calcd for ($C_{15}H_{13}N_3O_3S$): 315.0678; found 315.0670.

**1.16 5-(Pyridin-2-yl)-1*H*-pyrazol-3-yl 4-nitrobenzenesulfonate 19c**: 77% yield, mp (recrystallized from $CH_2Cl_2$) 224.5–225.5 °C; $^1$H NMR (DMSO, 400 MHz) $\delta$ 8.60 (d, $J$ = 4.4 Hz, 1 H, H-6), 8.46 (d, $J$ = 8.8 Hz, 2 H, 2 × ArH), 8.23 (d, $J$ = 8.8 Hz, 2 H, 2 × ArH), 7.91–7.87 (m, 1 H, H-3), 7.84–7.82 (m, 1 H, H-5), 7.39–7.36 (m, 1 H, H-4), 6.74 (s, 1 H, H-4′); $^{13}$C NMR (DMSO, 100 MHz) $\delta$ 153.42, 151.07, 149.46, 146.87, 143.18, 139.85, 137.52, 130.09, 124.93, 123.79, 120.32, 94.41; IR (neat) 3690 (m), 1549 (s), 1439 (s), 1321 (s), 1218 (s), 1137 (m), 1082 (m), 960 (m) cm$^{-1}$; ESIMS calcd for ($C_{14}H_{10}N_4O_5S$): 346.0372; found 346.0370.

**1.17 5-(Pyridin-2-yl)-1*H*-pyrazol-3-yl 2-nitrobenzenesulfonate 19d**: 80% yield, mp (recrystallized from $CH_2Cl_2$) 233.5–234.5 °C; $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 8.51–8.49 (m, 1 H, H-6), 8.10–8.08 (m, 1 H, ArH), 7.83-7.66 (m, 4 H, H-4 + 3 × ArH), 7.54–7.52 (m, 1 H, H-3), 7.23–7.21 (m, 1 H, H-5), 6.51 (s, 1 H, H-4′); $^{13}$C NMR (DMSO, 100 MHz) $\delta$ 153.35, 149.49, 147.84, 146.87, 143.18, 137.55, 136.83, 133.19, 131.74, 126.99, 125.39, 123.81, 120.29, 94.46; IR (neat) 3681 (m), 1554 (s), 1440 (s), 1375 (s), 1223 (s), 1161 (m), 1051 (m), 955 (m) cm$^{-1}$; ESIMS calcd for ($C_{14}H_{10}N_4O_5S$): 346.0372; found 346.0376.

**1.18 5-(Pyridin-2-yl)-1*H*-pyrazol-3-yl 4-fluorobenzenesulfonate 19e**: 76% yield, mp (recrystallized from $CH_2Cl_2$) 224.1–225.4 °C; $^1$H NMR (*d*-Acetone, 400 MHz) $\delta$ 8.59 (d, $J$ = 4.4 Hz, 1 H, H-6), 8.06–8.02 (m, 2 H, 2 × ArH), 7.90-7.81 (m, 2 H, H-3 + H-4), 7.47–7.42 (m, 2 H, 2 × ArH), 7.37–7.35 (m, 1 H, H-5), 6.67 (s, 1 H, H-4′); $^{13}$C NMR (*d*-Acetone, 100 MHz) $\delta$ 157.81, 151.50, 149.22, 143.10, 139.28, 136.68, 134.64, 132.26, 131.14, 125.57, 121.98, 96.19; IR (neat) 3698 (m), 1523 (s), 1448 (s), 1399 (s), 1229 (s), 1159 (m), 1058 (m), 930 (m) cm$^{-1}$; ESIMS calcd for ($C_{14}H_{10}FN_3O_3S$): 319.0427; found 319.0423.

**1.19 5-(Pyridin-2-yl)-1*H*-pyrazol-3-yl 4-bromobenzenesulfonate 19f**: 83% yield, mp (recrystallized from $CH_2Cl_2$) 227.6–228.2 °C; $^1$H NMR (*d*-Acetone, 400 MHz) $\delta$ 8.60–8.58 (m, 1 H, H-6), 7.89–7.87 (m, 4 H, 4 × ArH), 7.86–7.85 (m, 1 H, H-4), 7.83–7.82 (m, 1 H, H-3), 7.37–7.34 (m, 1 H, H-5), 6.68 (s, 1 H, H-4′); $^{13}$C NMR (*d*-Acetone, 100 MHz) $\delta$ 156.91, 150.40, 148.12, 142.00, 138.18, 135.58, 133.54, 131.16, 130.04, 124.47, 120.98, 95.09; IR (neat) 3681 (m), 1554 (s), 1440 (s), 1375 (s), 1223 (s), 1161 (m), 1051 (m), 955 (m) cm$^{-1}$; ESIMS calcd for ($C_{14}H_{10}BrN_3O_3S$): 378.9626; found 378.9621.

**1.20 5-(Pyridin-2-yl)-1*H*-pyrazol-3-yl 4-methoxybenzenesulfonate 19g**: 85% yield, mp (recrystallized from $CH_2Cl_2$) 220.5–221.8 °C; $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 8.56 (d, $J$ = 4.8 Hz, 1 H, H-6), 7.84 (d, $J$ = 9.2 Hz, 2 H, 2 × ArH), 7.75 (t, $J$ = 7.6 Hz, 1 H, H-4), 7.54 (d, $J$ = 8.0 Hz, 1 H, H-3), 7.25–7.22 (m, 1 H, H-5), 6.93 (d, $J$ = 9.2 Hz, 2 H, 2 × ArH), 6.50 (s, 1 H, H-4′), 3.82 (s, 3 H, $OCH_3$); $^{13}$C NMR ($CDCl_3$, 100 MHz) $\delta$ 164.19, 154.75, 149.13, 146.69, 142.27, 136.78, 131.50, 130.87, 130.79, 123.61, 120.42, 114.56, 94.60, 55.65 ($OCH_3$); IR (neat) 3613 (m), 1554 (s), 1440 (s), 1375 (s), 1223 (s), 1161 (m), 1051 (m), 955 (m) cm$^{-1}$; ESIMS calcd for ($C_{15}H_{13}N_3O_4S$): 331.0627; found 331.0622.

**1.21 5-(Pyridin-2-yl)-1*H*-pyrazol-3-yl methanesulfonate 19h**: 74% yield, mp

(recrystallized from $CH_2Cl_2$) 223.1–224.5 °C; $^1$H NMR (*d*-Acetone, 400 MHz) $\delta$ 8.64–8.63 (m, 1 H, H-6), 7.92–7.85 (m, 2 H, H-3 + H-4), 7.40–7.37 (m, 1 H, H-5), 6.78 (s, 1 H, H-4′), 3.43 (s, 3 H, $CH_3$); $^{13}$C NMR ($CDCl_3$, 100 MHz) $\delta$ 159.19, 154.93, 150.36, 144.43, 137.83, 123.90, 121.49, 96.49, 37.98 ($CH_3$); IR (neat) 3719 (m), 1220 (s), 1135 (m), 1059 (m), 935 (m) cm$^{-1}$; ESIMS calcd for ($C_9H_9N_3O_3S$): 239.0365; found 239.0364.

## 2 Biology

### 2.1 Cell culture and reagents

HepG2 2.2.15 cells derived from HepG2 human hepatocellular carcinoma cells and stably transfected with a head-to-tail HBV DNA dimer[3] were maintained in MEM with heat-inactivated 10% fetal bovine serum (FBS) and 1% antibiotics and grown at 37°C in a humidified atmosphere of 5% and 95% air. The synthesized compounds were dissolved in DMSO (Sigma-Aldrich). The final concentration of DMSO in all reactions was maintained at 0.1% in all experiments.

### 2.2 Cell viability assay

The cytotoxic effect of compounds was determined by a CellTiter 96® $AQ_{ueous}$ one solution cell proliferation assay kit (MTS) (Promega) to pinpoint the non-toxic test compound concentration in HepG2 2.2.15 cells. Briefly, HepG2 2.2.15 cells were plated into 96-well plates at a density of $4 \times 10^4$cells /ml for 24 h. The cells were then treated with serial dilutions of compounds for 3 days, and the toxicity of cells was measured according to the manufacturer's protocol. All measurements were performed in four replicates, and the results are presented as relative percentages over that of the control group.

### 2.3 Determination of viral HBsAg and HBeAg antigens

After treating the HepG2 2.2.15 cells cells, the levels of the viral surface antigen (HBsAg)–and e antigen (HBeAg) were measured in the culture media using an

enzyme immunoassay (EIA) kit (Johnson and Johnson), according to the manufacturer's instructions.

### 2.4 Real-time PCR analysis of HBV DNA level during treatment

The quantity of viral DNA in the medium was determined by Real-time PCR analysis by using NucleoSpin Blood mini-kit (Macherey-Nagel) for viral DNA extraction. The forward primer was 5′-AGGAGGCTGTAGGCATAAATTGG-3′ and the reverse primer was 5′-CAGCTTGGAGGCTTGAACAGT-3′[4] were synthesized for PCR to detected viral genome. The PCR reactions were performed using SYBR Green PCR master mix and the primer pair with the following program: initial denaturation at 50°C for 2 min and 95°C for 10 min, followed by 45 cycles of amplification at 95°C for 15s and annealing/extending at 58°C for 1 min.[5]

### 2.5 Statistical Analysis and Quantification of data

The data were expressed as the mean and the standard deviation (SD) of the mean from three independent experiments using GraphPad Prism (GraphPad Software Inc.). Variance analysis and the Student's *t*-test were used for data analysis. Differences were considered significant when $P < 0.05$. Quantitative data from Northern blot analysis were obtained using the computing densitometer and TotalLab Quant software (Nonlinear Dynamics Ltd.).

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