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Synthesis and Cytotoxicity of Novel Hexahydroquinoline-benzenesulfonamide Derivatives

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ABSTRACT

*The starting enamine derivatives **1a,c** were allowed to react with different substituted benzylidenemalononitrile derivatives **2a,b** to afford the corresponding tetrahydroquinoline-o-aminocarbonitrile derivatives **4a-d**. The treatment of **4a-d** with conc. H_2SO_4 at room temperature led to formation of hexahydroquinoline-o-aminocarboxamide derivatives **5a-d**, while complete hydrolysis had been occurred upon their refluxing with conc. H_2SO_4 to get the acid analogues **6a-d**. Condensation reaction of the derivatives **4a-d** with various acid chlorides in pyridine afforded the corresponding hexahydroquinoline-acetamide/benzamide derivatives **7a-d** & **8a-d**. Furthermore, the compounds **4a-d** were fused with urea, thiourea, formamide and acetic anhydride to gain the novel hexa(tetra)hydropyrimido[4,5-b]quinolin derivatives **9a-d**, **10a-d**, **11a-d** and **12a-d**, respectively. Some of the newly synthesized analogues were chosen to evaluate their in-vitro cytotoxic activity against human liver carcinoma cell lines (HEPG2). The obtained data revealed that the tested derivatives produced significant activity in comparison with the used reference drug Doxorubicin.*

Keywords: Hexahydroquinoline, benzenesulfonamides, carbonic anhydrase enzyme, liver carcinoma cell lines, cytotoxic activity.

INTRODUCTION

Carbonic anhydrases (CAs; also known as carbonate dehydratases EC 4.2.1.1) are ubiquitous metalloenzymes present in prokaryotes and eukaryotes. Basically, there are several cytosolic forms (CA-I, CA-II, CA-III, and CA-VII), four membrane_bound forms (CA-IV, CA-IX, CA-XII, and CA-XIV), one mitochondrial form (CA-V), as well as a secreted CA form (CA-VI) [1-3]. They all catalyze a very simple physiological reaction, the interconversion between carbon dioxide and the bicarbonate ion ($CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$). The catalytic domain of CAs contains an active site Zn^{+2}. This metal cation is a strong Lewis acid that binds to and activates a substrate H_2O molecule to catalyzes the reversible hydration reaction of carbon dioxide. Thus, CAs are involved in crucial physiological processes connected with respiration and transport of CO_2/bicarbonate between metabolizing tissues and the lungs, pH and CO_2 homeostasis, electrolyte secretion in a variety of tissues/organs, biosynthetic reactions (such as the gluconeogenesis, lipogenesis, and ureagenesis), bone resorption, calcification, tumorigenicity, and many other physiologic or pathologic processes [4]. For decades, inhibitors of CA have been a mainstay of human clinical intervention for a range of diseases, however more recently a role for CA inhibition as an anticancer therapy has been identified owing to the over expression of some CA isoforms in cancer cells and a minimal expression in normal tissue. So, this enzyme family is one of the most important therapeutical targets in the treatment of cancer disease through reducing the provision of bicarbonate for the synthesis of nucleotides and other cell components such as membrane lipids [5,6].

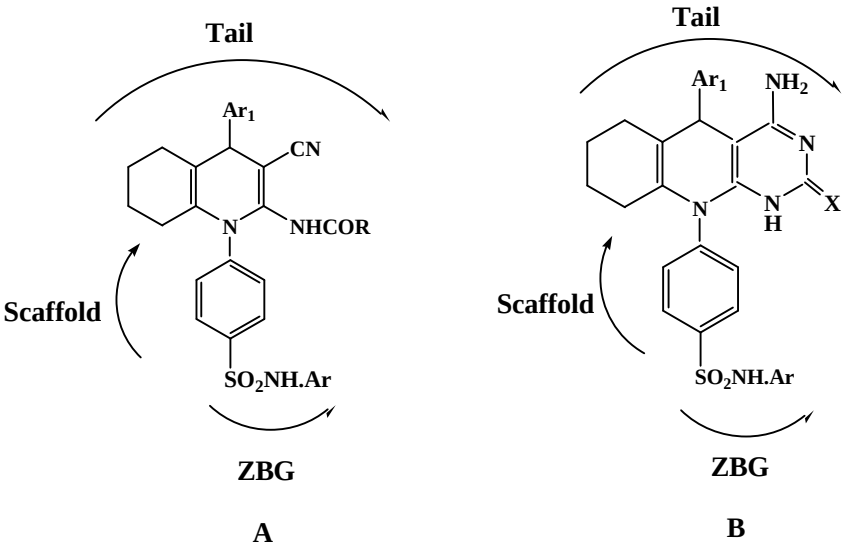
Sulfonamides are known to possess anticancer activity through different mechanisms of actions [7-9] and it was reported that their high affinity for CAs is the most prominent mechanism for their antitumor activity [10]. Such

compounds possess the anion ($ArSO_2NH^-$) that is a zinc binding group (ZBG) by which they interact with the metal ion in the active site of the enzyme either by substituting the non protein zinc ligand to generate a tetrahedral adduct or by addition to the metal coordination sphere to generate a trigonal bipyramidal species [3,11,12]. This aromatic sulfonamide group has served as a very reliable anchor upon which medicinal chemists have appended 'tails' to deliver inhibitors with improved potency and desirable selectivity profiles [13].

Quinolines, hydroquinolines and hexahydropyrimido[4,5-b]quinolins are important building blocks in different compounds exhibiting efficient anticancer potency [14-17]. Also, combination of several quinoline derivatives with sulfonamide moiety has been reported to have significant anticancer activity [18,19].

In the view of the above mentioned points, and in the light of the combination among quinolines and hydroquinolines with sulfonamide moieties might produce synergistic CA inhibition effect, the target of the present investigation was synthesis of novel derivatives bearing sulfonamide moiety conjugated to quinoline and pyrimido quinoline rings and some of them were selected as representative examples to evaluate their cytotoxic activity against human liver carcinoma cell lines (HEPG2).

The synthesized derivatives were designed to comply with pharmacophore of the compounds that may act as CA inhibitors. The most general structure features of a CAI include: a zinc binding group (ZBG) which corresponds to SO_2NH^- group; the sulfonamide is attached to a scaffold which is usually a benzene ring; a tail attached to the scaffold which corresponds to the side chain that possess a hydrophilic link that binds with the hydrophilic part of the active site and a hydrophobic link that can interact with the hydrophobic part of the active site of the enzyme. The general formulae of the prepared analogues are A, B [1]:



MATERIALS AND METHODS

Chemistry

All melting points are uncorrected and were recorded on an open glass capillary tubes using an Electrothermal IA 9100 digital melting point apparatus. Elemental microanalyses were carried out at Micro analytical Unit, Central Services Laboratory, National Research Center, Dokki, Cairo, Egypt, using Vario Elementar and were found within ±0.5% of the theoretical values. Infrared spectra were recorded on a Jasco FT/IR-6100, Fourier transform, Infrared spectrometer (Japan) at cm^{-1} scale using KBr disc technique at Central Services Laboratory, National Research Center, Dokki, Cairo, Egypt. ^{1}H-NMR spectra were determined by using a JEOl EX-270 NMR spectrometer (Japan) at Central Services Laboratory, National Research Center, Dokki, Cairo, Egypt. The mass spectra were measured with a Finnigan MAT SSQ-7000 mass spectrometer at Central Services Laboratory, Cairo University, Giza, Cairo, Egypt. Follow up of the reactions and checking the purity of the compounds were made by TLC on silica gel-precoated aluminium sheets (Type 60, F 254, Merck, Darmstadt, Germany) and the spots were detected by exposure to UV analysis lamp at λ 254/366 nm for few seconds.

General procedure for synthesis of enamine compounds 1a,c

A solution mixture of cyclohexanone (1 mL, 0.01 mol) and the appropriate sulfa drug namely; sulfapyridine and sulfadiazine (0.01 mol) in glacial acetic acid (30 mL) was refluxed for 6 h. Upon cooling, the solution was poured

onto ice/water and the obtained solid was filtered and re-crystallized from ethanol to get the desired enamines **1a,c** respectively.

3-(Cyclohexenylamino)-*N*-(pyridin-2-yl)benzenesulfonamide (**1a**)
Yield (80%); mp 187 ˚C; IR (KBr) ν_{max}/cm^{-1}: 3443, 3345 (2NH), 3012 (CH, aromatic), 2998 (CH, aromatic), 1360, 1123 (SO_2.NH); ^{1}H-NMR (DMSO-d_6 , δ ppm): 1.72-1.96 (m, 9H, 4CH_2, CH, cyclohexyl), 7.11-7.83 (m, 8H, aromatic) 9.21, 9.53 (2s, 2NH, exchangeable with D_2O); MS *m/z (%)*: 329 ($M^{\cdot+}$, 44). Anal. Calcd for $C_{17}H_{19}N_3O_2S$ (329.12): C, 61.98; H, 5.81; N, 12.76; S, 9.73. Found: C, 61.71; H, 5.54; N, 13.00; S, 9.43.

3-(Cyclohexenylamino)-*N*-(pyrimidin-2-yl)benzenesulfonamide (**1c**)
Yield (82%); mp 220 ˚C; IR (KBr) ν_{max}/cm^{-1}: 3445, 3352 (2NH), 3010 (CH, aromatic), 2998 (CH, aromatic), 1346, 1153 (SO_2.NH); ^{1}H-NMR (DMSO-d_6, δ ppm): 1.75-2.01 (m, 9H, 4CH_2, CH, cyclohexyl), 7.21-7.91 (m, 7H, aromatic) 9.42, 9.71 (2s, 2NH, exchangeable with D_2O); MS *m/z (%)*: 329 ($M^{\cdot+}$, 35); MS *m/z (%)*: 330 ($M^{\cdot+}$, 32). Anal. Calcd for $C_{16}H_{18}N_4O_2S$ (330.4): C, 58.16; H, 5.49; N, 16.96; S, 9.70. Found: C, 58.00; H, 5.21; N, 16.62; S, 9.93.

General procedure for synthesis of tetrahydroquinoline-*o*-aminocarbonitrile derivatives 4a-d
A solution mixture of the compounds **1a,c** (0.01 mol) and different substituted benzylidenemalononitrile derivatives namely: 2-(4-hydroxy-3-methoxybenzylidene)malononitrile and 2-(4-chlorobenzylidene)malononitrile (0.01 mol) in absolute ethyl alcohol (30 mL) containing triethylamine (1 mL) was refluxed for 6 h. After the reaction completion, the excess solvent was evaporated under reduced pressure and the precipitated solid was collected by filtration and re- crystallized by ethanol to give the desired derivatives **4a-d**.

4-(2-Amino-3-cyano-4-(4-hydroxy-3-methoxyphenyl)-5,6,7,8-tetrahydroquinolin-1(4*H*)-yl)-*N*-(pyridin-2-yl)benzenesulfonamide (4a)
Yield (72%); mp 174-176 ˚C; IR (KBr) ν_{max}/cm^{-1}: 3545-3210 (OH, NH, NH_2), 2210 (CN), 1313, 1142 (SO_2.NH); ^{1}H NMR (DMSO-d_6, δ ppm): 1.68-1.90 (m, 8H, 4CH_2, cyclohexyl), 3.85 (s, 3H, OCH_3), 4.82 (s, 2H, NH_2, exchangeable with D_2O), 5.10 (s, 1H, CH, pyridine), 7.11-8.11 (m, 11H, aromatic) 9.51, 10.23 (2s, 2H, NH, OH, exchangeable with D_2O); MS *m/z (%)*: 529 (M^+, 32). Anal. Calcd for $C_{28}H_{27}N_5O_4S$ (529.61): C, 63.50; H, 5.14; N, 13.22; S, 6.05. Found: C, 63.23; H, 5.00; N, 12.92; S, 6.38.

4-(2-Amino-4-(4-chlorophenyl)-3-cyano-5,6,7,8-tetrahydroquinolin-1(4*H*)-yl)-*N*-(pyridin-2-yl) benzenesulfonamide (4b)
Yield (72%); mp 179-181 ˚C; IR (KBr) ν_{max}/cm^{-1}: 3445, 3338, 3210 (NH, NH_2), 2210 (CN), 1313, 1150 (SO_2.NH); ^{1}H NMR (DMSO-d_6, δ ppm): 1.61-1.82 (m, 8H, 4CH_2, cyclohexyl), 4.82 (s, 2H, NH_2, exchangeable with D_2O), 5.00 (s, 1H, CH, pyridine), 7.21-8.0 (m, 12H, aromatic), 9.01 (s, 1H, NH, exchangeable with D_2O); MS *m/z (%)*: 518 (M^+, 20), 520 (M^++2, 7) . Anal. Calcd for $C_{27}H_{24}ClN_5O_2S$ (518.03): C, 62.60; H, 4.67; Cl, 6.84; N, 13.52; S, 6.19. Found: C, 62.37; H, 4.23; Cl, 7.12; N, 13.73; S, 6.37.

4-(2-Amino-3-cyano-4-(4-hydroxy-3-methoxyphenyl)-5,6,7,8-tetrahydroquinolin-1(4*H*)-yl)-*N*-(pyrimidin-2-yl) benzenesulfonamide (4c)
Yield (79%); mp 194-196 ˚C; IR (KBr) ν_{max}/cm^{-1}: 3525-3290 (OH, NH, NH_2), 2218 (CN), 1320, 1156 (SO_2.NH); ^{1}H NMR (DMSO-d_6, δ ppm): 1.58-1.79 (m, 8H, 4CH_2, cyclohexyl), 3.80 (s, 3H, OCH_3), 4.57 (s, 2H, NH_2, exchangeable with D_2O), 5.51 (s, 1H, CH, pyridine), 7.19-8.11 (m, 10H, aromatic), 9.40, 10.12 (s, 2H, NH, OH, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, δ ppm): 23.1, 24.3, 24.5, 26.2 (4CH_2, cyclohexyl), 45.4 (CH), 60.3 (OCH_3), 117.3 (CN), 110.3, 111.3, 112.1, 124.7, 126.3, 127.3, 128.5, 129.3, 135.6, 140.7, 142.3, 144.6, 151.5, 157.3, 167.2, 169.5 (aromatic-C); MS *m/z (%)*: 530 (M^+, 40). Anal. Calcd for $C_{27}H_{26}N_6O_4S$ (530.62): C, 61.12; H, 4.94; N, 15.84; S, 6.04. Found: C, 60.82; H, 5.11; N, 15.53; S, 6.34.

4-(2-Amino-4-(4-chlorophenyl)-3-cyano-5,6,7,8-tetrahydroquinolin-1(4*H*)-yl)-*N*-(pyrimidin-2-yl) benzenesulfonamide (4d)
Yield (68%); mp 184-186 ˚C; IR (KBr) ν_{max}/cm^{-1}: 3450, 3340, 3290 (NH, NH_2), 2215 (CN), 1320, 1156 (SO_2.NH); ^{1}H NMR (DMSO-d_6, δ ppm): 1.65-1.83 (m, 8H, 4CH_2, cyclohexyl), 4.77 (s, 2H, NH_2,exchangeable with D_2O), 5.11 (s, 1H, CH, pyridine), 7.01-8.00 (m, 10H, aromatic), 9.40, 10.12 (2s, 2H, NH, OH, exchangeable with D_2O);
MS *m/z (%)*: 518 (M^+, 42), 520 (M^++2, 15). Anal. Calcd for $C_{26}H_{23}ClN_6O_2S$ (519.02): C, 60.17; H, 4.47; Cl, 6.83; N, 16.19; S, 6.18. Found: C, 60.36; H, 4.81; Cl, 7.01; N, 16.35; S, 5.88.

General procedure for synthesis of hexahydroquinoline-*o*-aminocarboxamide compounds 5a-d
A solution of compounds **4a-d** (0.01 mol) in conc. H_2SO_4 (20 mL) was stirred at room temperature for 5 h. The obtained solid was collected and re-crystallized from ethanol to give the carboxamide derivatives **5a-d**.

2-Amino-4-(4-hydroxy-3-methoxyphenyl)-1-(4-(*N*-pyridin-2-ylsulfamoyl)phenyl)-1,4,5,6,7,8-hexahydro quinoline-3-carboxamide (5a)
Yield (68%); mp 217-219 ˚C; IR (KBr) ν_{max}/cm^{-1}: 3550-3290 (OH, NH, $2NH_2$), 1645 (C=O), 1343, 1123 (SO_2.NH); ^{1}H NMR (DMSO-d_6, δ ppm): 1.75-2.03 (m, 8H, $4CH_2$, cyclohexyl), 3.75 (s, 3H, OCH_3), 4.59 (s, 2H, NH_2, exchangeable with D_2O), 5.11 (s, 1H, CH, pyridine), 5.97 (s, 2H, -$CONH_2$, exchangeable with D_2O), 7.22-7.89 (m, 11H, aromatic), 9.40, 10.12 (2s, 2H, NH, OH, exchangeable with D_2O) ; ^{13}C NMR (DMSO-d_6, δ ppm): 23.1, 24.3, 24.5, 26.2 ($4CH_2$, cyclohexyl), 45.4 (CH), 60.3 (OCH_3), 117.3 (CN), 110.5, 111.3, 113.5, 124.7, 125.4, 126.3, 128.0, 128.5, 129.3, 136.6, 140.7, 142.3, 145.7, 154.5, 157.3, 167.7, 170.5 (aromatic-C); MS *m/z (%)*: 547 (M^+, 34). Anal. Calcd for $C_{28}H_{29}N_5O_5S$ (547.63): C, 61.41; H, 5.34; N, 12.79; S, 5.86. Found: C, 61.00; H, 5.51; N, 13.12; S, 5.65.

2-Amino-4-(4-chlorophenyl)-1-(4-(*N*-pyridin-2-ylsulfamoyl)phenyl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxamide (5b)
Yield (72%); mp 220-222 ˚C; IR (KBr) ν_{max}/cm^{-1}: 3450-3290 (NH, $2NH_2$), 1655 (C=O), 1333, 1145 (SO_2.NH); ^{1}H NMR (DMSO-d_6, δ ppm): 1.75-2.11 (m, 8H, $4CH_2$, cyclohexyl), 4.89 (s, 2H, NH_2, exchangeable with D_2O), 5.62 (s, 1H, CH, pyridine), 6.11 (s, 2H, -$CONH_2$, exchangeable with D_2O), 7.22-7.89 (m, 12H, aromatic), 9.01 (s, 1H, NH, exchangeable with D_2O); MS *m/z (%)*: 536 (M^+, 43), 538 (M^+ +2, 15). Anal. Calcd for $C_{27}H_{26}ClN_5O_3S$ (536.05): C, 60.50; H, 4.89; N, 13.06; S, 5.98. Found: C, 60.32; H, 4.54; N, 13.41; S, 6.22.

2-Amino-4-(4-hydroxy-3-methoxyphenyl)-1-(4-(*N*-pyrimidin-2-ylsulfamoyl)phenyl)-1,4,5,6,7,8-hexahydro quinoline-3-carboxamide (5c)
Yield (65%); mp 197-199 ˚C; IR (KBr) ν_{max}/cm^{-1}: 3550-3290 (OH, NH, $2NH_2$), 1658 (C=O), 1340, 1120 (SO_2.NH); ^{1}H NMR (DMSO-d_6, δ ppm): 1.65-1.83 (m, 8H, $4CH_2$, cyclohexyl); 3.81 (s, 3H, OCH_3), 4.67 (s, 2H, NH_2,exchangeable with D_2O), 5.32 (s, 1H, CH, pyridine), 6.41 (s, 2H, -$CONH_2$, exchangeable with D_2O), 7.22-7.89 (m, 10H, aromatic), 9.01, 10.32 (2s, 2H, NH, OH, exchangeable with D_2O); MS *m/z (%)*: 548 (M^+, 15). Anal. Calcd for $C_{27}H_{28}N_6O_5S$ (548.61): C, 59.11; H, 5.14; N, 15.32; S, 5.84. Found: C, 58.86; H, 5.54; N, 15.00; S, 5.61.

2-Amino-4-(4-chlorophenyl)-1-(4-(*N*-pyrimidin-2-ylsulfamoyl)phenyl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxamide (5d)
Yield (69%); mp 207-209 ˚C; IR (KBr) ν_{max}/cm^{-1}: 3448-3238 (NH, $2NH_2$), 1656 (C=O), 1345, 1135 (SO_2.NH); ^{1}H NMR (DMSO-d_6, δ ppm): 1.65-1.86 (m, 8H, $4CH_2$, cyclohexyl), 4.73 (s, 2H, NH_2, exchangeable with D_2O), 5.62 (s, 1H, CH, pyridine), 6.11 (s, 2H, -$CONH_2$, exchangeable with D_2O), 7.29-8.01 (m, 11H, aromatic), 9.21 (s, 1H, NH, exchangeable with D_2O); MS *m/z (%)*: 537 (M^+, 25), 539 (M^+ +2, 9). Anal. Calcd for $C_{26}H_{25}ClN_6O_3S$ (537.03): C, 58.15; H, 4.69; N, 15.65; S, 5.97. Found: C, 58.46; H, 4.54; N, 15.20; S, 5.61.

General procedure for synthesis of hexahydroquinoline-*o*-aminocarboxylic acid compounds 6a-d
A solution of compounds **4a-d** (0.01 mol) in conc. H_2SO_4 (20 mL) was refluxed for 2 h. The obtained solid was collected and crystallized from ethanol to give the carboxylic acid analogues **6a-d**, respectively.

2-Amino-4-(4-hydroxy-3-methoxyphenyl)-1-(4-(*N*-pyridin-2-ylsulfamoyl)phenyl)-1,4,5,6,7,8-hexahydro quinoline-3-carboxylic acid (6a)
Yield (60%); mp 282-284 ˚C; IR (KBr) ν_{max}/cm^{-1}: 3560-3238 (2OH, NH, NH_2), 1702 (C=O), 1350, 1160 (SO_2.NH); ^{1}H NMR (DMSO-d_6, δ ppm): 1.71-1.93 (m, 8H, $4CH_2$, cyclohexyl), 3.75 (s, 3H, OCH_3), 5.11 (s, 2H, NH_2, exchangeable with D_2O), 5.62 (s, 1H, CH, pyridine), 7.01-8.01 (m, 11H, aromatic), 9.21, 10.21, 10.51 (3s, 3H, NH, 2OH, exchangeable with D_2O); MS *m/z (%)*: 548 (M^+, 54). Anal. Calcd for $C_{28}H_{28}N_4O_6S$ (548.61): C, 61.30; H, 5.14; N, 10.21; S, 5.84. Found: C, 61.51; H, 5.42; N, 9.82; S, 6.12.

2-Amino-4-(4-chlorophenyl)-1-(4-(*N*-pyridin-2-ylsulfamoyl)phenyl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylic acid (6b)
Yield (63%); mp 254-256 ˚C; IR (KBr) ν_{max}/cm^{-1}: 3560-3238 (OH, NH, NH_2), 1698 (C=O), 1352, 1165 (SO_2.NH); ^{1}H NMR (DMSO-d_6, δ ppm): 1.71-1.93 (m, 8H, $4CH_2$, cyclohexyl), 4.91 (s, 2H, NH_2, exchangeable with D_2O), 5.43 (s, 1H, CH, pyridine), 7.11-8.21 (m, 12H, aromatic), 9.21, 10.31 (2s, 2H, NH, OH, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, δ ppm): 22.2, 24.7, 25.4, 26.9 ($4CH_2$, cyclohexyl), 32.8 (CH), 111.4, 112.9, 113.3, 116.4, 128.1, 128.7, 129.4, 130.8, 131.4, 138.8, 140.6, 144.5, 148.4, 153.6, 163.1 (aromatic-C), 171.4 (COOH); MS *m/z (%)*: 537 (M^+, 20), 539 (M^+ +2, 8). Anal. Calcd $C_{27}H_{25}ClN_4O_4S$ (537.03): C, 60.39; H, 4.69; N, 10.43; S, 5.97. Found: C, 60.13; H, 4.98; N, 10.19; S, 5.50.

2-Amino-4-(4-hydroxy-3-methoxyphenyl)-1-(4-(*N*-pyrimidin-2-ylsulfamoyl)phenyl)-1,4,5,6,7,8-hexahydro quinoline-3-carboxylic acid (6c)
Yield (62%); mp 274-276 ˚C; IR (KBr) ν_{max}/cm^{-1}: 3558-3240 (2OH, NH, NH_2), 1700 (C=O), 1350, 1160 (SO_2.NH); ^{1}H NMR (DMSO-d_6, δ ppm): 1.71-1.90 (m, 8H, $4CH_2$, cyclohexyl), 3.81 (s, 3H, OCH_3), 5.21 (s, 2H, NH_2, exchangeable with D_2O), 5.90 (s, 1H, CH, pyridine), 7.01-8.01 (m, 10H, aromatic), 8.90, 10.00, 10.51 (3s, 3H, NH,

2OH, exchangeable with D_2O); MS *m/z (%)*: 548 (M^+-1, 64). Anal. Calcd for $C_{27}H_{27}N_5O_6S$ (549.6): C, 59.00; H, 4.95; N, 12.74; S, 5.83. Found: C, 59.32; H, 5.24; N, 12.53; S, 5.62.

2-Amino-4-(4-chlorophenyl)-1-(4-(*N*-pyrimidin-2-ylsulfamoyl)phenyl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylic acid (6d)

Yield (63%); mp 258-260 ˚C; IR (KBr) ν_{max}/cm^{-1}: 3560-3238 (OH, NH, NH_2), 1705 (C=O), 1352, 1165 (SO_2.NH); ^{1}H NMR (DMSO-d_6, δ ppm): 1.75-1.89 (m, 8H, $4CH_2$, cyclohexyl), 5.12 (s, 2H, NH_2, exchangeable with D_2O), 6.01 (s, 1H, CH, pyridine), 7.20-8.22 (m, 11H, aromatic), 9.21, 10.21 (2s, 2H, OH, exchangeable with D_2O); MS *m/z (%)*: 538 (M^+, 53), 540 (M^+ +2, 18). Anal. Calcd $C_{26}H_{24}ClN_5O_4S$ (538.02): C, 58.04; H, 4.50; N, 13.02; S, 5.96. Found: C, 57.84; H, 4.82; N, 13.41; S, 6.34.

General procedure for synthesis of hexahydroquinoline-acetamide & hexahydroquinoline-benzamide compounds 7a-d & 8a-d

A mixture of derivatives **4a-d** (0.01 mol) and different acid chloride derivatives namely: acetyl chloride and benzoyl chloride (0.01 mol) in pyridine (20 mL) was refluxed for 5 h. The reaction mixture was cooled and poured onto cold water, then acidified by diluted HCl. The solid obtained was filtered and crystallized from dioxane to give the derivatives **7a-d & 8a-d** respectively.

N-(3-cyano-4-(4-hydroxy-3-methoxyphenyl)-1-(4-(*N*-pyridin-2-ylsulfamoyl)phenyl)-1,4,5,6,7,8-hexahydro quinolin-2-yl)acetamide (7a)

Yield (72%); mp 223-225 ˚C; IR (KBr) ν_{max}/cm^{-1}: 3534-3357 (OH, 2NH), 2210 (CN), 1656 (C=O), 1356, 1143 (SO_2.NH); ^{1}H NMR (DMSO-d_6, δ ppm): 1.21 (s, 3H, CH_3), 1.73-2.00 (m, 8H, $4CH_2$, cyclohexyl), 3.80 (s, 3H, OCH_3), 5.65 (s, 1H, CH, pyridine), 7.23-8.00 (m, 11H, aromatic), 9.41, 9.62, 10.51 (3s, 3H, 2NH, OH, exchangeable with D_2O); MS *m/z (%)*: 571 (M^+, 35). Anal. Calcd $C_{30}H_{29}N_5O_5S$ (571.65) : C, 63.03; H, 5.11; N, 12.25; S, 5.61. Found: C, 63.32; H, 5.42; N, 12.02; S, 5.91.

N-(4-(4-chlorophenyl)-3-cyano-1-(4-(*N*-pyridin-2-ylsulfamoyl)phenyl)-1,4,5,6,7,8-hexahydroquinolin-2-yl) acetamide (7b)

Yield (63%); mp 198-200 ˚C; IR (KBr) ν_{max}/cm^{-1}: 3456, 3351 (2NH), 2218 (CN), 1654 (C=O), 1352, 1165 (SO_2.NH); ^{1}H NMR (DMSO-d_6, δ ppm): 1.21 (s, 3H, CH_3), 1.68-1.90 (m, 8H, $4CH_2$, cyclohexyl), 5.84 (s, 1H, CH, pyridine), 7.10-8.11 (m, 12H, aromatic), 9.32, 9.65 (2s, 2H, 2NH, exchangeable with D_2O); MS *m/z (%)*: 559 (M^+, 30), 561(M^+ +2, 10). Anal. Calcd $C_{29}H_{26}ClN_5O_3S$ (560.07): C, 62.19; H, 4.68; N, 12.50; S, 5.73. Found: C, 62.45; H, 4.37; N, 12.12; S, 6.03.

N-(3-cyano-4-(4-hydroxy-3-methoxyphenyl)-1-(4-(*N*-pyrimidin-2-ylsulfamoyl)phenyl)-1,4,5,6,7,8-hexahydro quinolin-2-yl)acetamide (7c)

Yield (71%); mp 275-277 ˚C; IR (KBr) ν_{max}/cm^{-1}: 3534-3357 (OH, 2NH), 2220 (CN), 1666 (C=O), 1356, 1120 (SO_2.NH); ^{1}H NMR (DMSO-d_6, δ ppm): 1.23 (s, 3H, CH_3), 1.73-1.86 (m, 8H, $4CH_2$, cyclohexyl), 3.84 (s, 3H, OCH_3), 5.55 (s, 1H, CH, pyridine), 7.23-8.00 (m, 10H, aromatic), 9.56, 9.62, 10.43 (3s, 3H, 2NH, OH, exchangeable with D_2O); MS *m/z (%)*: 572 (M^+, 28). Anal. Calcd $C_{29}H_{28}N_6O_5S$ (572.63) : C, 60.83; H, 4.93; N, 14.68; S, 5.60 . Found: C, 61.21; H, 5.32; N, 14.34; S, 5.23.

N-(4-(4-chlorophenyl)-3-cyano-1-(4-(*N*-pyrimidin-2-ylsulfamoyl)phenyl)-1,4,5, 6,7,8-hexahydroquinolin-2-yl) acetamide (7d)

Yield (69%); mp 234-236 ˚C; IR (KBr) ν_{max}/cm^{-1}: 3450, 3354 (2NH), 2218 (CN), 1664 (C=O), 1360, 1140 (SO_2.NH); ^{1}H NMR (DMSO-d_6, δ ppm): 1.30 (s, 3H, CH_3), 1.75-1.90 (m, 8H, $4CH_2$, cyclohexyl), 5.81 (s, 1H, CH, pyridine), 7.10-8.09 (m, 11H, aromatic), 9.41, 9.65 (2s, 2H, 2NH, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, δ ppm): 21.3, 23.3, 24.4, 24.9, 26.3 ($4CH_2$, cyclohexyl, CH_3), 45.2 (CH), 117.5 (CN), 110.1, 110.9, 111.3, 116.3, 128.1, 128.8, 129.4, 130.5, 131.3, 140.0, 140.5, 144.1, 154.6, 157.8, 166.9 (aromatic-C), 169.3 (C=O); MS *m/z (%)*: 560 (M^+, 45), 562 (M^+ +2, 18). Anal. Calcd $C_{28}H_{25}ClN_6O_3S$ (561.05): C, 59.94; H, 4.49; N, 14.98; S, 5.72. Found: C, 59.53; H, 4.21; N, 15.13; S, 5.41.

N-(3-cyano-4-(4-hydroxy-3-methoxyphenyl)-1-(4-(*N*-pyridin-2-ylsulfamoyl)phenyl)-1,4,5,6,7,8-hexahydro quinolin-2-yl)benzamide (8a)

Yield (72%); mp 244-246 ˚C; IR (KBr) ν_{max}/cm^{-1}: 3556-3343 (OH, 2NH), 2215 (CN), 1650 (C=O), 1356, 1140 (SO_2.NH); ^{1}H NMR (DMSO-d_6, δ ppm): 1.69-2.00 (m, 8H, $4CH_2$, cyclohexyl), 3.75 (s, 3H, OCH_3), 6.10 (s, 1H, CH, pyridine), 7.23-8.21 (m, 16H, aromatic), 9.32, 9.54, 10.51 (3s, 3H, 2NH, OH, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, δ ppm): 23.1, 24.3, 24.5, 26.2 ($4CH_2$, cyclohexyl), 45.4 (CH), 56.3 (OCH_3), 117.2 (CN), 110.6, 111.4, 113.1, 114.5, 116.1, 116.7, 122.3, 127.3, 128.1, 128.9. 129.9, 132.4, 135.5, 138.6, 140.7, 142.1, 144.5, 148.6, 152.1,

153.4, 154.3 (aromatic-C), 164.1 (C=O); MS *m/z (%)*: 633 (M^+, 37). Anal. Calcd $C_{35}H_{31}N_5O_5S$ (633.72) : C, 66.33; H, 4.93; N, 11.05; S, 5.06. Found: C, 66.12; H, 4.59; N, 11.34; S, 4.87.

N-(4-(4-chlorophenyl)-3-cyano-1-(4-(*N*-pyridin-2-ylsulfamoyl)phenyl)-1,4,5,6,7,8-hexahydroquinolin-2-yl) benzamide (8b)

Yield (70%); mp 210-212 ˚C; IR (KBr) ν_{max}/cm^{-1}: 3440, 3289 (2NH), 2220 (CN), 1655 (C=O), 1354, 1165 (SO_2.NH); ^{1}H NMR (DMSO-d_6, δ ppm): 1.70-1.90 (m, 8H, $4CH_2$, cyclohexyl), 6.14 (s, 1H, CH, pyridine), 7.22-8.15 (m, 17H, aromatic), 9.50, 9.65 (2s, 2H, 2NH, exchangeable with D_2O); MS *m/z (%)*: 622 (M^+, 30), 624 (M^+ +2, 10). Anal. Calcd $C_{34}H_{28}ClN_5O_3S$ (622.14): C, 65.64; H, 4.54; N, 11.26; S, 5.15. Found: C, 65.42; H, 4.27; N, 11.51; S, 5.49.

N-(3-cyano-4-(4-hydroxy-3-methoxyphenyl)-1-(4-(*N*-pyrimidin-2-ylsulfamoyl)phenyl)-1,4,5,6,7,8-hexahydro quinolin-2-yl)benzamide (8c)

Yield (78%); mp 263-265 ˚C; IR (KBr) ν_{max}/cm^{-1}: 3535-3357 (OH, 2NH), 2220 (CN), 1656 (C=O), 1356, 1135 (SO_2.NH); ^{1}H NMR (DMSO-d_6, δ ppm): 1.77-1.98 (m, 8H, $4CH_2$, cyclohexyl), 3.70 (s, 3H, OCH_3), 6.23 (s, 1H, CH, pyridine), 7.01-8.00 (m, 15H, aromatic), 9.39, 9.71, 10.52 (3s, 3H, 2NH, OH, exchangeable with D_2O); MS *m/z (%)*: 634 (M^+, 15). Anal. Calcd $C_{34}H_{30}N_6O_5S$ (634.72) : C, 64.34; H, 4.76; N, 13.24; S, 5.05. Found: C, 64.63; H, 5.02; N, 12.93; S, 5.38.

N-(4-(4-chlorophenyl)-3-cyano-1-(4-(*N*-pyrimidin-2-ylsulfamoyl)phenyl)-1,4,5,6,7,8-hexahydroquinolin-2-yl) benzamide (8d)

Yield (70%); mp 236-238 ˚C; IR (KBr) ν_{max}/cm^{-1}: 3459, 3320 (2NH), 2210 (CN), 1664 (C=O), 1360, 1140 (SO_2.NH); ^{1}H NMR (DMSO-d_6, δ ppm): 1.75-2.00 (m, 8H, $4CH_2$, cyclohexyl), 6.12 (s, 1H, CH, pyridine), 7.25-8.12 (m, 16H, aromatic), 9.23, 9.65 (2s, 2H, 2NH, exchangeable with D_2O); MS *m/z (%)*: 623 (M^+, 15), 625 (M^+ +2, 5). Anal. Calcd $C_{33}H_{27}ClN_6O_3S$ (623.12): C, 63.61; H, 4.37; N, 13.49; S, 5.15. Found: C, 63.23; H, 4.19; N, 13.87; S, 5.38.

General procedure for synthesis of the compounds 2-oxo/ 2-thioxo-hexahydro pyrimido[4,5-*b*]quinolin derivatives 9a-d & 10a-d

A mixture of compounds **4a-d** (0.01 mol) and urea or thiourea (0.01 mol) was fused at 220˚C for 1 h. Then the reaction mixture was triturated with ethanol. The obtained solid was crystallized from dioxane to give the desired derivatives **9a-d** and **10a-d**, respectively.

4-(4-Amino-5-(4-hydroxy-3-methoxyphenyl)-2-oxo-1,2,6,7,8,9-hexahydro pyrimido[4,5-*b*]quinolin-10(5*H*)-yl)-*N*-(pyridin-2-yl)benzenesulfonamide (9a)

Yield (73%); mp 198-200 ˚C; IR (KBr) ν_{max}/cm^{-1}: 3548-3254 (OH, 2NH, NH_2), 1700 (C=O), 1356, 1138 (SO_2.NH); ^{1}H NMR (DMSO-d_6, δ ppm): 1.69-1.89 (m, 8H, $4CH_2$, cyclohexyl), 3.81 (s, 3H, OCH_3), 4.96 (s, 2H, NH_2,exchangeable with D_2O), 5.60 (s, 1H, CH, pyridine), 7.21-7.89 (m, 11H, aromatic), 9.21, 9.63, 10.51 (3s, 3H, 2NH, OH, exchangeable with D_2O); MS *m/z (%)*: 572 (M^+, 67). Anal. Calcd for $C_{29}H_{28}N_6O_5S$ (572.63): C, 60.83; H, 4.93; N, 14.68; S, 5.60. Found: C, 61.13; H, 4.62; N, 14.31; S, 5.90.

4-(4-Amino-5-(4-chlorophenyl)-2-oxo-1,2,6,7,8,9-hexahydropyrimido[4,5-*b*] quinolin-10(5*H*)-yl)-*N*-(pyridin-2-yl)benzenesulfonamide (9b)

Yield (77%); mp 190-192 ˚C; IR (KBr) ν_{max}/cm^{-1}: 3448-3235 (2NH, NH_2), 1689 (C=O), 1370, 1140 (SO_2.NH); ^{1}H NMR (DMSO-d_6, δ ppm): 1.61-1.91 (m, 8H, $4CH_2$, cyclohexyl), 4.56 (s, 2H, NH_2, exchangeable with D_2O), 5.65 (s, 1H, CH, pyridine), 7.21-8.00 (m, 12H, aromatic), 9.21, 9.68 (2s, 2H, 2NH, exchangeable with D_2O); MS *m/z (%)*: 561 (M^+, 68), 563 (M^+ +, 23). Anal. Calcd for $C_{28}H_{25}ClN_6O_3S$ (561.06): C, 59.94; H, 4.49; N, 14.98; S, 5.72. Found: C, 60.24; H, 4.17; N, 14.53; S, 6.02.

4-(4-Amino-5-(4-hydroxy-3-methoxyphenyl)-2-oxo-1,2,6,7,8,9-hexahydro pyrimido[4,5-*b*]quinolin-10(5*H*)-yl)-*N*-(pyrimidin-2-yl)benzenesulfonamide (9c)

Yield (69%); mp 217-219 ˚C; IR (KBr) ν_{max}/cm^{-1}: 3565-3210 (OH, 2NH, NH_2), 1718 (C=O), 1360, 1140 (SO_2.NH); ^{1}H NMR (DMSO-d_6, δ ppm): 1.71-1.99 (m, 8H, $4CH_2$, cyclohexyl), 3.82 (s, 3H, OCH_3), 5.51 (s, 2H, NH_2, exchangeable with D_2O), 5.87 (s, 1H, CH, pyridine), 7.21-8.00 (m, 10H, aromatic), 9.42, 9.66, 10.34 (3s, 3H, 2NH, OH, exchangeable with D_2O); MS *m/z (%)*: 573 (M^+, 25). Anal. Calcd for $C_{28}H_{27}N_7O_5S$ (573.63): C, 58.63; H, 4.74; N, 17.09; S, 5.59. Found: C, 58.51; H, 4.42; N, 17.40; S, 5.99.

4-(4-Amino-10-(4-chlorophenyl)-2-oxo-1,2,6,7,8,9-hexahydropyrimido[5,4-*b*]quinolin-5(10*H*)-yl)-*N*-(pyrimidin-2-yl)benzenesulfonamide (9d)
Yield (78%); mp 206-208˚C; IR (KBr) ν_{max}/cm^{-1}: 3465-3256 (2NH, NH_2), 1718 (C=O), 1360, 1140 (SO_2.NH); ^1H NMR (DMSO-d_6, δ ppm): 1.71 -1.99 (m, 8H, $4CH_2$, cyclohexyl), 5.51 (s, 2H, NH_2, exchangeable with D_2O), 5.87 (s, 1H, CH, pyridine), 7.21-8.00 (m, 11H, aromatic), 9.42, 9.66 (2s, 2H, 2NH, exchangeable with D_2O); MS *m/z (%)*: 562 (M^+, 17), 564 (M^+ +2, 7). Anal. Calcd for $C_{27}H_{24}ClN_7O_3S$ (562.04): C, 57.70; H, 4.30; N, 17.44; S, 5.71. Found: C, 58.04; H, 4.18; N, 17.70; S, 5.45.

4-(4-Amino-5-(4-hydroxy-3-methoxyphenyl)-2-thioxo-1,2,6,7,8,9-hexahydro pyrimido[4,5-*b*]quinolin-10(5*H*)-yl)-*N*-(pyridin-2-yl)benzenesulfonamide (10a)
Yield (81%); mp 280-282 ˚C; IR (KBr) ν_{max}/cm^{-1}: 3569-3226 (OH, 2NH, NH_2), 1343, 1120 (SO_2.NH), 1210 (C=S); ^1H NMR (DMSO-d_6, δ ppm): 1.65-1.80 (m, 8H, $4CH_2$, cyclohexyl), 3.75 (s, 3H, OCH_3), 4.96 (s, 2H, NH_2, exchangeable with D_2O), 5.50 (s, 1H, CH, pyridine), 7.11-7.80 (m, 11H, aromatic), 9.27, 9.50, 10.61 (3s, 3H, 2NH, OH, exchangeable with D_2O); MS *m/z (%)*: 588 (M^+, 60). Anal. Calcd for $C_{29}H_{28}N_6O_4S_2$ (588.71): C, 59.17; H, 4.79; N, 14.28; S, 10.89. Found: C, 59.51; H, 4.97; N, 14.06; S, 11.27.

4-(4-Amino-5-(4-chlorophenyl)-2-thioxo-1,2,6,7,8,9-hexahydropyrimido[4,5-*b*]quinolin-10(5*H*)-yl)-*N*-(pyridin-2-yl)benzenesulfonamide (10b)
Yield (83%); mp 275-277 ˚C; IR (KBr) ν_{max}/cm^{-1}: 3452-3259 (2NH, NH_2), 1345, 1135 (SO_2.NH), 1228 (C=S); ^1H NMR (DMSO-d_6, δ ppm): 1.71-1.97 (m, 8H, $4CH_2$, cyclohexyl), 4.83 (s, 2H, NH_2, exchangeable with D_2O), 5.12 (s, 1H, CH, pyridine), 7.32-8.10 (m, 12H, aromatic), 9.21, 9.68 (2s, 2H, 2NH, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, δ ppm): 22.2, 24.7, 25.4, 26.9 (4CH2, cyclohexyl), 41.1 (CH), 107.1, 110.3, 111.1, 116.3, 128.1, 128.8, 129.4, 130.5, 131.0, 140.3, 144.6, 156.4, 157.1, 162.3,169.0 (aromatic-C); MS *m/z (%)*: 577 (M^+, 76), 579 (M^+ + 2, 25). Anal. Calcd for $C_{28}H_{25}ClN_6O_2S_2$ (577.12): C, 58.27; H, 4.37; N, 14.56; S, 11.11. Found C, 58.56; H, 4.12; N, 14.28; S, 11.50.

4-(4-Amino-5-(4-hydroxy-3-methoxyphenyl)-2-thioxo-1,2,6,7,8,9-hexahydro pyrimido[4,5-*b*]quinolin-10(5*H*)-yl)-*N*-(pyrimidin-2-yl)benzenesulfonamide (10c)
Yield (79%); mp 277-279 ˚C; IR (KBr) ν_{max}/cm^{-1}: 3545-3239 (OH, 2NH, NH_2), 1360, 1140 (SO_2.NH), 1220 (C=S); ^1H NMR (DMSO-d_6, δ ppm): 1.72-2.02 (m, 8H, $4CH_2$, cyclohexyl), 3.82 (s, 3H, OCH_3), 4.97 (s, 2H, NH_2,exchangeable with D_2O), 5.80 (s, 1H, CH, pyridine), 7.05-8.00 (m, 10H, aromatic), 9.51, 9.73, 10.56 (3s, 3H, 2NH, OH, exchangeable with D_2O); MS *m/z (%)*: 589 (M^+, 37). Anal. Calcd for $C_{28}H_{27}N_7O_4S_2$ (589.69): C, 57.03; H, 4.62; N, 16.63; S, 10.88. Found: C, 57.45; H, 4.34; N, 16.92; S, 11.18.

4-(4-Amino-5-(4-chlorophenyl)-2-thioxo-1,2,6,7,8,9-hexahydropyrimido[4,5-*b*]quinolin-10(5*H*)-yl)-*N*-(pyrimidin-2-yl)benzenesulfonamide (10d)
Yield (78%); mp 256-258 ˚C; IR (KBr) ν_{max}/cm^{-1}: 3465-3250 (2NH, NH_2), 1356, 1137 (SO_2.NH), 1210 (C=S); ^1H NMR (DMSO-d_6, δ ppm): 1.71-2.00 (m, 8H, $4CH_2$, cyclohexyl), 5.62 (s, 2H, NH_2, exchangeable with D_2O), 6.00 (s, 1H, CH, pyridine), 7.21-8.00 (m, 11H, aromatic), 9.52, 9.81 (2s, 2H, 2NH, exchangeable with D_2O); MS *m/z (%)*: 578 (M^+, 23), 580 (M^+ +2, 7). Anal. Calcd for $C_{27}H_{24}ClN_7O_2S_2$ (578.11): C, 56.09; H, 4.18; N, 16.96; S, 11.09. Found: C, 56.32; H, 4.34; N, 17.21; S, 10.89.

General procedure for synthesis of tetrahydropyrimido[4,5-*b*]quinolin compounds 11a-d
A solution of the compounds **4a-d** (0.01 mol) in formamide (30 mL) was refluxed for 6 h. Then, the reaction mixture was cooled and poured onto ice/water. The precipitated solid was collected by filtration and re crystallized from dioxane to get the desired derivatives **11a-d**.

4-(4-Amino-5-(4-hydroxy-3-methoxyphenyl)-6,7,8,9-tetrahydropyrimido[4,5-*b*]quinolin-10(5*H*)-yl)-*N*-(pyridin-2-yl)benzenesulfonamide (11a)
Yield (83%); mp 215-217 ˚C; IR (KBr) ν_{max}/cm^{-1}: 3550-3243 (OH, NH, NH_2), 1343, 1120 (SO_2.NH); ^1H NMR (DMSO-d_6, δ ppm): 1.71 -1.98 (m, 8H, $4CH_2$, cyclohexyl), 3.75 (s, 3H, OCH_3), 4.91 (s, 2H, NH_2, exchangeable with D_2O), 5.71 (s, 1H, CH, pyridine), 7.31-8.21 (m, 12H, aromatic), 9.40, 10.52 (2s, 2H, NH, OH, exchangeable with D_2O); MS *m/z (%)*: 556 (M^+, 60). Anal. Calcd for $C_{29}H_{28}N_6O_4S$ (556.65): C, 62.57; H, 5.07; N, 15.10; S, 5.76. Found: C, 62.61; H, 5.37; N, 15.34; S, 5.51.

4-(4-Amino-5-(4-chlorophenyl)-6,7,8,9-tetrahydropyrimido[4,5-*b*]quinolin-10(5*H*)-yl)-*N*-(pyridin-2-yl)benzenesulfonamide (11b)
Yield (85%); mp 211-213 ˚C; IR (KBr) ν_{max}/cm^{-1}: 3452-3259 (NH, NH_2), 1366, 1146 (SO_2.NH); ^1H NMR (DMSO-d_6, δ ppm): 1.68-1.93 (m, 8H, $4CH_2$, cyclohexyl), 5.11 (s, 2H, NH_2,exchangeable with D_2O), 5.71 (s, 1H, CH, pyridine), 7.12-8.16 (m, 13H, aromatic), 9.21 (s, 1H, NH, exchangeable with D_2O); MS *m/z (%)*: 545 (M^+, 65), 547

(M^+ + 2, 22). Anal. Calcd for $C_{28}H_{25}ClN_6O_2S$ (545.06): C, 61.70; H, 4.62; N, 15.42; S, 5.88. Found: C, 61.43; H, 4.30; N, 15.81; S, 6.10.

4-(4-Amino-5-(4-hydroxy-3-methoxyphenyl)-6,7,8,9-tetrahydropyrimido[4,5-*b*]quinolin-10(5*H*)-yl)-*N*-(pyrimidin-2-yl)benzenesulfonamide (11c)

Yield (82%); mp 243-245 ˚C; IR (KBr) ν_{max}/cm^{-1}: 3553-3230 (OH, NH, NH_2), 1360, 1140 (SO_2.NH); ^{1}H NMR (DMSO-d_6, δ ppm): 1.72-1.98 (m, 8H, $4CH_2$, cyclohexyl), 3.85 (s, 3H, OCH_3), 4.61 (s, 2H, NH_2, exchangeable with D_2O), 5.80 (s, 1H, CH, pyridine), 7.21-8.00 (m, 11H, aromatic), 9.51, 10.56 (2s, 2H, NH, OH, exchangeable with D_2O); MS *m/z (%)*: 557.62 (M^+, 56). Anal. Calcd for $C_{28}H_{27}N_7O_4S$ (557.62): C, 60.31; H, 4.88; N, 17.58; S, 5.75. Found: C, 60.52; H, 5.12; N, 17.23; S, 5.41.

4-(4-Amino-10-(4-chlorophenyl)-6,7,8,9-tetrahydropyrimido[5,4-*b*]quinolin-10(5*H*)-yl)-*N*-(pyrimidin-2-yl)benzenesulfonamide (11d)

Yield (85%); mp 232-234 ˚C; IR (KBr) ν_{max}/cm^{-1}: 3445-3235 (NH, NH_2), 1360, 1140 (SO_2.NH); ^{1}H NMR (DMSO-d_6, δ ppm): 1.71 -1.96 (m, 8H, $4CH_2$, cyclohexyl), 5.61 (s, 2H, NH_2, exchangeable with D_2O), 6.42 (s, 1H, CH, pyridine), 7.23-8.00 (m, 12H, aromatic), 9.52 (s, 1H, NH, exchangeable with D_2O); MS *m/z (%)*: 546 (M^+, 28), 548 (M^+ +2, 9). Anal. Calcd for $C_{27}H_{24}ClN_7O_2S$ (546.04): C, 59.39; H, 4.43; N, 17.96; S, 5.87. Found: C, 59.71; H, 4.62; N, 18.21; S, 5.54.

General procedure for synthesis of hexahydropyrimido[4,5-*b*]quinolin compounds 12a-d

A solution of the compounds **4a-d** (0.01 mol) in acetic anhydride (30 mL) was refluxed for 8 h. Then, the reaction mixture was concentrated under reduced pressure and the obtained solid was collected and re-crystallized from ethanol to get the desired derivatives **12a-d**.

4-(5-(4-hydroxy-3-methoxyphenyl)-2-methyl-4-oxo-3,4,6,7,8,9-hexahydro pyrimido[4,5-*b*]quinolin-10(5*H*)-yl)-*N*-(pyridin-2-yl)benzenesulfonamide (12a)

Yield (85%); mp 273-275 ˚C; IR (KBr) ν_{max}/cm^{-1}: 3550-3343 (OH, 2NH), 1689 (C=O), 1333, 1156 (SO_2.NH), ^{1}H NMR (DMSO-d_6, δ ppm): 1.21 (s, 3H, CH_3), 1.68-1.92 (m, 8H, $4CH_2$, cyclohexyl); 3.85 (s, 3H, OCH_3), 5.71 (s, 1H, CH, pyridine), 7.31-8.21 (m, 11H, aromatic), 9.40, 9.62, 10.52 (3s, 3H, 2NH, OH, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, δ ppm): 22.2, 24.7, 25.0, 25.4, 26.9 ($4CH_2$, cyclohexyl, CH3), 40.1 (CH), 56.3 (OCH_3), 100.1, 111.2, 113.2, 114.4, 116.1, 123.3,128.9, 135.3, 138,6, 140.3, 142.2, 144.3, 148.4, 151.7, 152.3, 153.4, 162.2, 164.7 (aromatic-C); MS *m/z (%)*: 571 (M^+, 20). Anal. Calcd for $C_{30}H_{29}N_5O_5S$ (571.65): C, 63.03; H, 5.11; N, 12.25; S, 5.61. Found: C, 63.34; H, 5.42; N, 12.09; S, 5.41.

4-(5-(4-Chlorophenyl)-2-methyl-4-oxo-3,4,6,7,8,9-hexahydropyrimido[4,5-*b*]quinolin-10(5*H*)-yl)-*N*-(pyridin-2-yl)benzenesulfonamide (12b)

Yield (82%); mp 266-268 ˚C; IR (KBr) ν_{max}/cm^{-1}: 3452-3359 (2NH), 1702 (C=O), 1358, 1166 (SO_2.NH); ^{1}H NMR (DMSO-d_6, δ ppm): 1.24 (s, 3H, CH_3), 1.68-1.93 (m, 8H, $4CH_2$, cyclohexyl), 5.71 (s, 1H, CH, pyridine), 7.12-8.16 (m, 12H, aromatic), 9.21, 9.51 (s, 2H, 2NH, exchangeable with D_2O); MS *m/z (%)*: 560 (M^+, 75), 562 (M^+ +2, 25). Anal. Calcd for $C_{29}H_{26}ClN_5O_3S$ (560.07): C, 62.19; H, 4.68; N, 12.50; S, 5.73. Found: C, 62.34; H, 4.89; N, 12.34; S, 6.03.

4-(5-(4-hydroxy-3-methoxyphenyl)-2-methyl-4-oxo-3,4,6,7,8,9-hexahydro pyrimido[4,5-*b*]quinolin-10(5*H*)-yl)-*N*-(pyrimidin-2-yl)benzenesulfonamide (12c)

Yield (86%); mp 285-287 ˚C; IR (KBr) ν_{max}/cm^{-1}: 3540-3225 (OH, 2NH), 1690 (C=O), 1345, 1150 (SO_2.NH); ^{1}H NMR (DMSO-d_6, δ ppm): 1.23 (s, 3H, CH_3), 1.68-1.92 (m, 8H, $4CH_2$, cyclohexyl), 3.82 (s, 3H, OCH_3), 6.51 (s, 1H, CH, pyridine), 7.21-8.01 (m, 10H, aromatic), 9.42, 9.65, 10.52 (3s, 3H, 2NH, OH, exchangeable with D_2O); MS *m/z (%)*: 572 (M^+, 10). Anal. Calcd for $C_{29}H_{28}N_6O_5S$ (572.63): C, 60.83; H, 4.93; N, 14.68; S, 5.60. Found: 61.12; H, 5.16; N, 14.32; S, 5.43.

4-(5-(4-Chlorophenyl)-2-methyl-4-oxo-3,4,6,7,8,9-hexahydropyrimido[5,4-*b*]quinolin-5(10*H*)-yl)-*N*-(pyrimidin-2-yl)benzenesulfonamide (12d)

Yield (80%); mp 247-249 ˚C; IR (KBr) ν_{max}/cm^{-1}: 3430-3267 (2NH), 1708 (C=O), 1358, 1156 (SO_2.NH); ^{1}H NMR (DMSO-d_6, δ ppm): 1.30 (s, 3H, CH_3), 1.68-1.93 (m, 8H, $4CH_2$, cyclohexyl), 5.91 (s, 1H, CH, pyridine), 7.12-8.16 (m, 11H, aromatic), 9.24, 9.59 (2s, 2H, 2NH, exchangeable with D_2O); MS *m/z (%)*: 561 (M^+, 15), 563 (M^+ +2, 5). Anal. Calcd for $C_{28}H_{25}ClN_6O_3S$ (561.05): C, 59.94; H, 4.49; N, 14.98; S, 5.72. Found: C, 60.31; H, 4.83; N, 14.42; S, 5.32.

In-vitro Cytotoxic screening

Cell growth inhibition assay

Ten analogues **4a,b**, **6b,c**, **8a,b,c**, **9b,d**, **10b** were selected as representative examples to evaluate their *in-vitro* inhibitory effects against cellular proliferation in human cultured liver carcinoma cell lines using Doxorubicin as a reference drug.

Liver cancer cell lines (HEPG2) were obtained from Cell Bank in National Cancer Institute, Cairo, Egypt. The potential toxicity of the selected newly synthesized derivatives was done by SRB using the method Skehan *et al.* [20] as follows: Cells were plated in 96-multiwell plate (104 cells/ well) for 24 h before treatment with compounds to allow attachment of cell to the wall of the plate. Different concentrations of the compound under test (1, 2.5, 5 and 10 g/ml) were added to the cell monolayer triplicate wells which were prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 h at 37°C and in atmosphere of 5% CO2. After 48 h, cells were fixed, washed and stained with Sulfo-Rhodamine-B stain. Excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer. Color intensity was measured in an ELISA reader. Measurements were done six times and averaged. The relation between surviving fraction and drug concentration is plotted to get the survival curve of each tumor cell line after the specified compound.

RESULTS AND DISCUSSION

Chemistry

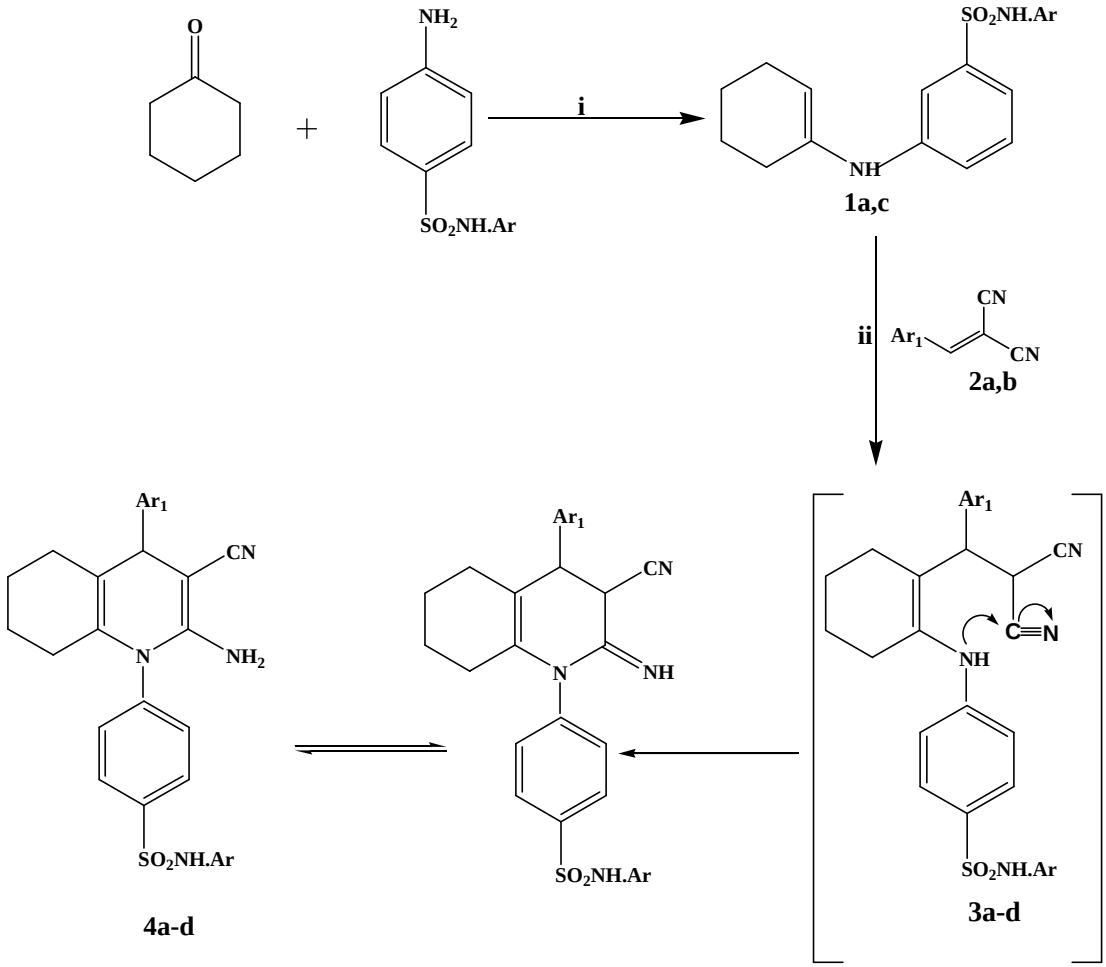
The preparation of our target compounds was started by the reaction of sulfa drugs namely: sulfapyridine and sulfadiazine with cyclohexanone in glacial acetic acid to get the enamine derivatives **1a,b** respectively. Treatment of **1a,b** with various substituted benzylidenemalononitrile derivatives namely; 2-(4-hydroxy-3-methoxybenzylidene)malononitrile and 2-(4-chlorobenzylidene)malononitrile **2a,b** respectively, prepared according the literature [21], in absolute ethanol containing a catalytic amount of triethylamine resulted in quinoline-o-aminocarbonitriles **4a-d** via the formation of the intermediate Michael type products **3a-d**, followed by intramolecular cyclization [22] (Scheme 1). The structures of all of the newly synthesized derivatives were established via the elemental analyses and IR, ^{1}H NMR and mass spectral data.

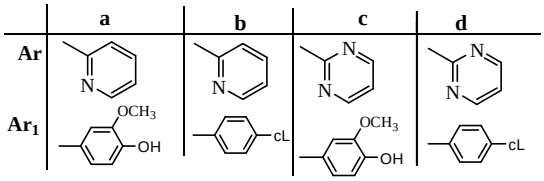
IR spectra of the compounds **4a-d** exhibited characteristic absorption bands at the range 3450-3210 and 2220-2210 cm^{-1} due to the respective NH, NH_2 and CN groups, while SO_2NH groups were represented as two absorption bands at the ranges 1370-1320 and 1180-1140 cm^{-1}. ^{1}H-NMR spectra of compounds **4a-d** (DMSO-d_6) revealed multiplet signals at δ 1.61-1.98 ppm due the presence of 8H of the cyclohexyl ring, in addition to two singlet signals at δ 4.57-4.82 ppm and 5.00-5.11 ppm representing 2H of NH_2 groups and the methine proton of the pyridine ring, respectively.

Stirring the derivatives **4a-d** with conc. H_2SO_4 at room temperature for 5 h furnished the hexahydroquinoline-*o*-aminocarboxamide derivatives **5a-d**, while upon their refluxing with conc. H_2SO_4 led to complete hydrolysis and the formation of the acid analogues **6a-d**. IR spectra of the carboxamide derivatives **5a-d** exhibited the disappearance of the characteristic band of CN group and showed the presence of stretch bands at the range 1645-1658 cm^{-1} corresponding to the carboxamide $CONH_2$ groups. Also, IR spectra of **6a-d** showed absorption bands at the range 1705-1698 cm^{-1} contributing the presence of the carboxylic COOH groups.

Furthermore, the reaction of **4a-d** compounds with different acid chloride derivatives namely; acetyl chloride and benzoyl chloride in pyridine was carried out in a trial for obtaining hexahydroquinoline-acetamide or benzamide derivatives **7a-d** & **8a-d**, respectively. IR spectra of compounds **7a-d** & **8a-d** revealed the presence of CN bands at the range 2210-2220 cm^{-1} and the amide C=O groups at the range 1666-1654 cm^{-1}. ^{1}H-NMR spectra of the acetamide derivatives **7a-d** represented singlet signals at δ 1.21-1.30 ppm due to the acetyl protons of COCH_3 groups.

Also, under condition of fusion of the derivatives **4a-d** with urea, thiourea and formamide, nucleophilic substitution has occurred followed by intramolecular cyclization to give the corresponding 2-oxo/ 2-thioxo-hexahydropyrimido[4,5-*b*]quinolin derivatives **9a-d** & **10a-d** and tetrahydropyrimido[4,5-*b*]quinolin compounds **11a-d**, respectively. IR spectra of the obtained compounds exhibited the disappearance of the absorption bands corresponding to CN groups and the appearance of absorption bands at the range 1718-1698 cm^{-1} due to the lactamic C=O in case of **9a-d** and at the range of 1228-1210 cm^{-1} due to C=S in case of **10a-d**. These IR data proved that cyclization process has occurred.

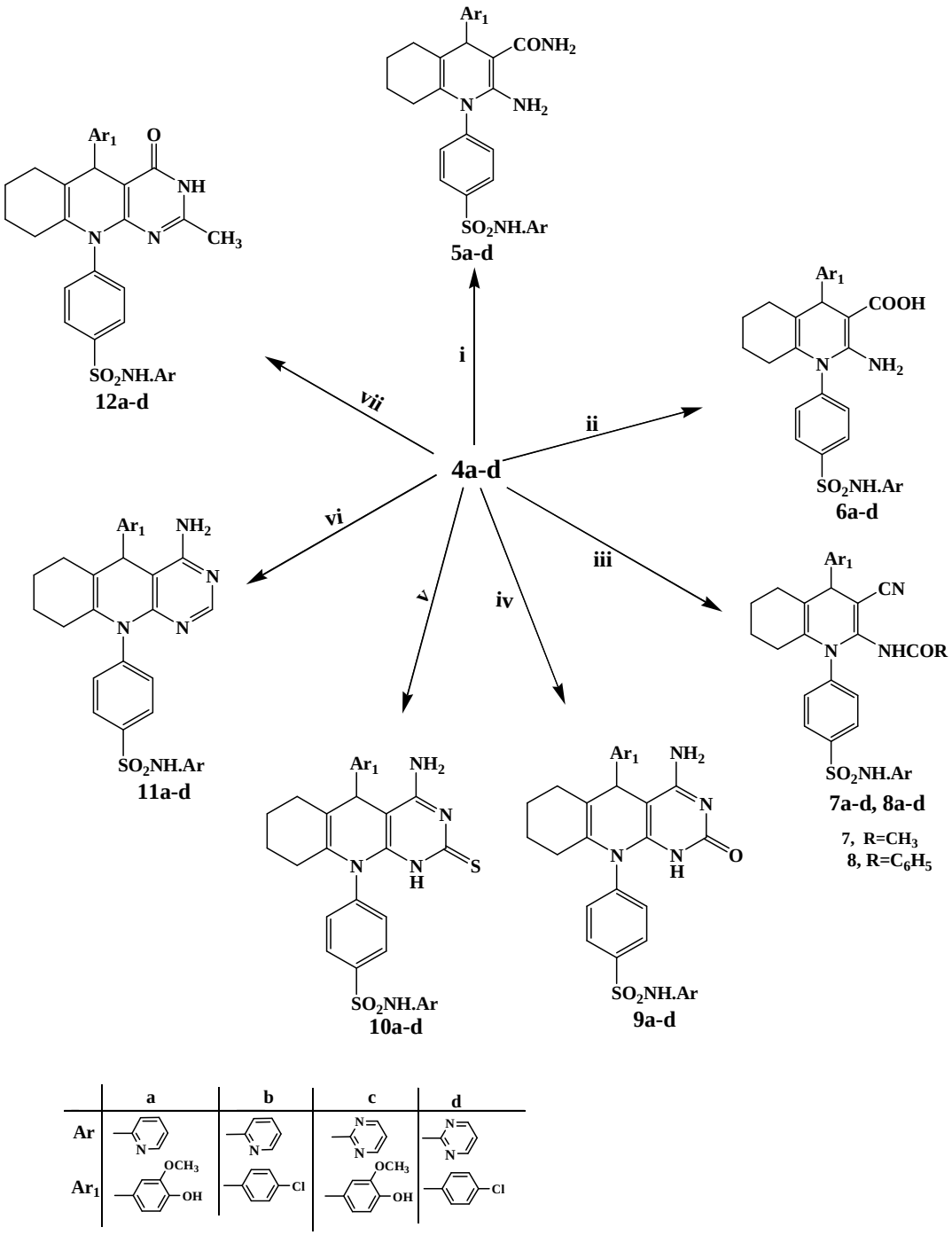




Reaction Conditions: i, glacial acetic acid, reflux for 3 h; ii, ethanol, triethylamine, reflux for 6h

Scheme 1

Further cyclization which underwent Dimroth rearrangement was performed via the reaction of the compounds **4a-d** with acetic anhydride under reflux for 8 h to produce hexahydropyrimido[4,5-*b*]quinolin compounds **12a-d**. IR spectra of the derivatives **12a-d** revealed the disappearance of the stretching bands of CN groups, in addition to the presence of absorption bands at the range of 1708-1689 cm^{-1} contributing to the lactamic C=O groups. Also, ^{1}H NMR spectra of **12a-d** showed siglet signals at 1.23 ppm representing 3H of the methyl groups at the 2-position of the hexahydropyrimido[4,5-*b*]quinolin nucleus (Scheme 2).



Reaction Conditions: i, conc. H_2SO_4, stirr at r.t. for 5 h; ii, conc. H_2SO_4, reflux for 5h; iii, pridine, reflux for 2 h; iv, v, urea/thiourea, fusion at 220 °C; vi,formamide, reflux for 6 h; vii, acetic anhydride, reflux for 8 h.

Scheme 2

Cytotoxic activity evaluation

In the present work, ten of the newly synthesized compounds **4a**, **4c**, **6b**, **6c**, **8a**, **8b**, **8d**, **9b**, **9d**, **10b** were selected to evaluate their *in- vitro* growth inhibitory activities against human cultured liver carcinoma cell lines (HEPG2) in comparison to Doxorubicin which is one of the most effective antitumor agents.

According to the resultant data (table 1), it is noteworthy that all of the tested derivatives produced growth inhibitory activity against the liver cancerous cells at IC_{50} approximately equal to or slightly less than that given by the reference drug. It has been noticed that the hexahydroquinoline-benzamide compounds **8a**, **8d**, **8b** and 2-thioxo-1,2,6,7,8,9-hexahydropyrimido[4,5-*b*]quinolin **10b** appeared to be the most potent agents (IC_{50}: 0.015-0.017µM). Slight reduction in the potency was observed when the *S* atom was replaced by *O* atom as the analogues 2-oxo-

1,2,6,7,8,9-hexahydropyrimido[4,5-*b*]quinolins **9b**, **9d** (IC_{50}: 0.019, 0.020 µM). Further decrease in the activity was exhibited by the parent tetrahydroquinoline-*o*-aminocarbonitrile **4a**, **4c** derivatives (IC_{50}: 0.022, 0.026 µM) and the hexahydroquinoline-*o*-aminocarboxylic acid derivatives **6c**, **6b** (IC_{50}: 0.021, 0.033 µM).

Table (1): The effect of some newly synthesized compounds against human liver carcinoma cell line (HEPG2)

Compounds	*IC_{50} (µM)*
4a	0.022
4c	0.026
6b	0.033
6c	0.020
8a	0.015
8b	0.017
8d	0.016
9b	0.019
9d	0.020
10b	0.017
Doxorubicin (Dox)	0.010

CONCLUSION

This work deals with synthesis of new different tetra(hexa)hydroquinoline-benzenesulfonamide derivatives. Ten compounds were selected as representatives to evaluate their cytotoxic potency. Based on the above data it may be worthwhile to deduce that the derivatives bearing the parent hexahydroquinoline-benzenesulfonamide-2-benzamide or 2-thioxo-hexahydropyrimido[4,5-*b*]quinolin-benzenesulfonamide nuclei produce potent cytotoxicity against the hepatic cancer cells which might be due to their suitable fitting and hydrogen bonds formation with the amino acids of carbonic anhydrase enzyme leading to its inhibition. This may contribute in part to their anticancer activity. These points might be taken in consideration while modifying novel hexahydroquinoline-benzenesulfonamide derivatives to optimize the cytotoxic potency.

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