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Synthesis and Evaluation of New Series of 1,4-Dihydropyridine Derivatives as Anticancer Agents

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Abstract

1,4-dihydropyridine derivatives represent one of the important classes of compounds possessing a wide variety of biological activities including anticancer activity. In the present study, (4-Alkyl/Aryl-1-substituted 2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxilicacid, 3,5-bis [2(aminothioxomethyl)hydrazides]) (6 a-l) were synthesized by the reaction of 4-alkyl/aryl-3,5-dicarboalkoxy-2,6-dimethyl-1,4-dihydropyridines (4 a-l) with thisemicarbazide and evaluated for their anti-cancer properties.

All the synthesized compounds were characterized by IR, NMR and Mass spectra and were screened to evaluating for anticancer activity against three cell lines (MCF-7, HeLa and Hep G_2) by using MTT assay method. The results showed that compounds 6j and 6l showed significant cytotoxicity with IC_{50} values ranging from 56µM - 74µM.

Keywords: Cancer; 1,4-Dihydropyridines; MTT assay; Thiosemicarbazide.

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1. Introduction

Chemotherapy plays an important role in the treatment of several cancers including breast cancer. However, during treatment with chemotherapeutic agents, the development of drug resistance is quite common in the metastatic cancers [1, 2]. After intermittent or prolonged exposure of tumor cells to only a single agent is often found to result in broad resistance to multiple structurally and functionally unrelated drugs [3]. Due to the development of multidrug resistance in cancer chemotherapy, necessity for effective therapy stimulated search for design and synthesis of newer agents. Hantzsch 1,4-dihydropyridines (1,4-DHPs) and their derivatives have acquired great importance in the area of organic and medicinal chemistry since they exhibit a fascinating array of pharmacological properties like antihypertensive [4,5], antimicrobial [6,7], anti-tubercular[8,9], antileishmanial [10, 11], antidyslipidemic [12] and cytotoxic activities [13,14]. 1,4-Dihydropyridines are commercially used as calcium channel blockers for the treatment of cardiovascular diseases. It is quite interesting to note that the 1,4-DHPs have gained prominence in the recent years for cancer chemotherapy, in addition to their calcium channel blocking action. 1,4-Dihydropyridines have been proved to be a new class of multidrug resistance (MDR) reversals in cancer treatment. Our team has been working in this area for the development of new 1,4-dihydropyridines as MDR reversal agents apart from anti-cancer and antitubercular activities [15-20]. Thosemicarbazide and semicarbazide derivatives have been documented as potential antibacterial, antifungal and antitubercular agents [21, 22]. Thiosemicarbazide derivatives were also reported to have significant antitumor [23, 24], anticoagulant [25] and antiviral properties [26].

In view of the wide range of biological properties associated with 1,4-dihydropyridines and thiosemicarbazide derivatives as anticancer agents, it was thought worthwhile to synthesize new molecules by incorporating thisemicarbazide and cyclohexyl/benzyl substitution with 1,4-dihydropyridines moieties in a single molecular framework and to evaluate their cytotoxic and antimicrobial activities.

2. Materials and Methods

Synthesis of all the compounds was carriedout by using Redleys reaction station. Melting points were determined in one-end-open capillary tubes on Toshniwal melting point apparatus and are uncorrected. IR spectra in KBr discs recorded on Perkin-Elmer BX-I series FT-IR spectrophotometer and ^{1}H NMR on a Bruker AVANCE-300 spectrometer. All NMR spectra were measured in $CDCl_3$ solution using tetra methylsilane as an internal standard and ^{1}H chemical shifts are reported as ppm. Mass spectra were recorded by using electro spray ionization technique (ESI) on the VG170708H mass spectrometer. Solvents were dried by conventional methods. Silica gel 60-120 mesh (Merck) was used as an adsorbent for column chromatography. TLC was performed on 5 –10 cm aluminum plates coated with silicagel 60F-254 (Merck) in an appropriate solvent.

2.1. Experiemental

2.1.1. 4-Alkyl/Aryl-1-substituted-3,5-bis(carboethoxy)-2,6-dimethyl-1,4-dihydropyridines

To the mixture of ethyl acetoacetate and different aliphatic and aromatic aldehydes in alcohol was added a solution of primary amine with shaking and the mixture was refluxed for 12 to 16 hours. Upon completion of

the reaction, alcohol was removed to the possible extent by rota evaporator and the residue was cooled and triturated with crushed ice, and thus resultant precipitate was filtered and washed with cold water and dried in vacuum.

2.1.2. 4-Alkyl/Aryl-1-substituted-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxilicacid-3,5-bis[2 (amino- -thioxomethyl)hydrazide]

A reaction mixture consisting of compound (4 a-l, 0.1 mol), thiosemicarbazide (0.2 mol) dissolved in ethanol (30 mL) and a few drops of DMSO was added. It was then heated under reflux for 10 h. The reaction mixture was cooled and then poured in to crushed ice. The separated solid was collected by filtration, washed with water and recrystallised using ethanol.

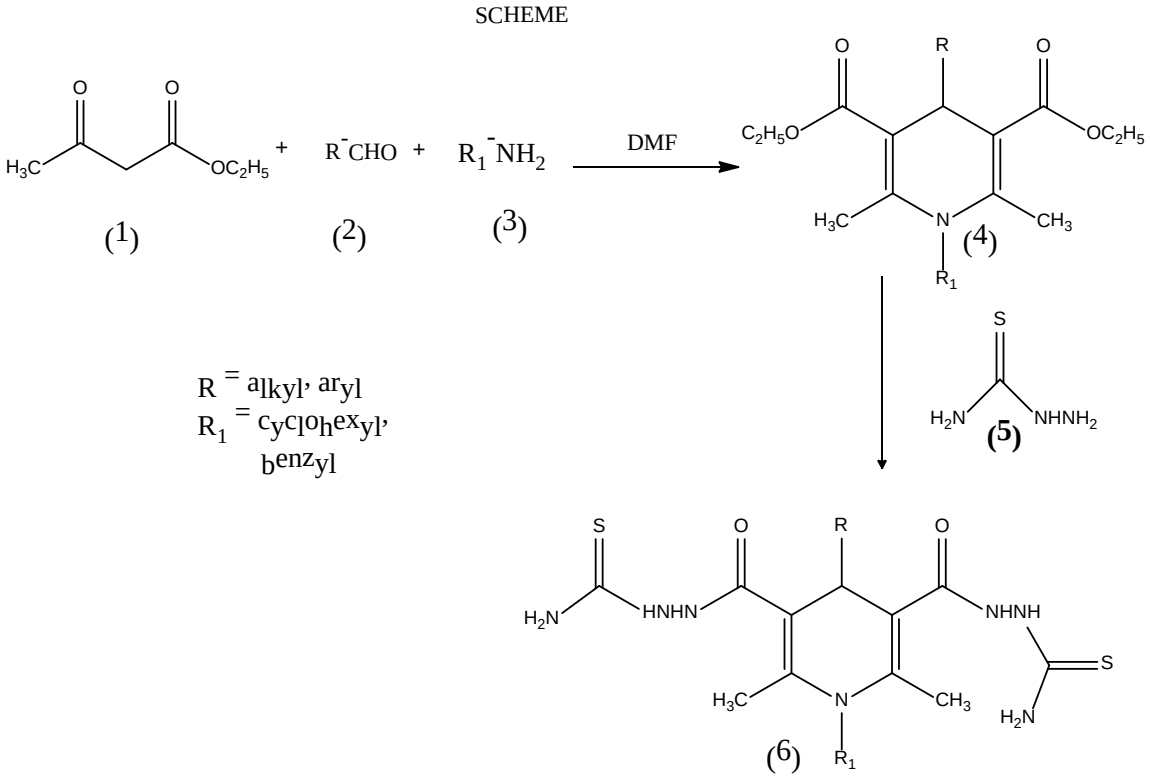

Figure 1: Scheme for the synthesis of 1,4-Dihydropyridine thiosemicarbazide derivatives.

2.2. Anticancer activity

Anticancer activity of the all the synthesized compounds was assessed by their cytotoxic effect against three cancer cell lines MCF-7, Hela, and Hep G2 (purchased from NCCS, Pune) using MTT assay method described by Mosmann and his colleagues [27]. The cells were grown in RPMI medium containing 10% FBS in humidified atmosphere of 5% CO_2 at 37 ^{o}C. Cells were trypsinized and seeded in 96 well plates at a density of 5000 cells per well and incubated for 24 hours to get monolayer of cells. Serial concentrations of compounds

were made with RPMI media using DMSO as co-solvent. After 24 hours the test molecules of various concentrations in fresh media were added to appropriate wells. Plates were incubated further for 48 hours and the assay was quenched 20 µl of (Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 5 mg/ml) and incubated for 60 min at 37^{o}C. The plates were dried and subsequently the dye bound to the cells was solubilised in 100 µl of DMSO. The spectrophotometric absorbance of the samples was observed using a microplate (ELISA) reader. The wavelength to measure absorbance of the formazan product was 570 nm on the ELISA reader (Thermo, USA).

3. Characterization

3.1. 2,2'-(1-cyclohexyl-2,6-dimethyl-4-phenyl-1,4-dihydropyridine-3,5-dicarbonyl) bis(hydrazinecarbothioamide) (6a)

IR (KBr, cm^{-1}): 3218 (NH_2), 3196(NH), 3043 (C-H aromatic), 1761 (C=O, Carbonyl); ^{1}H NMR (DMSO – d6) δ ppm: 0.97-1.27 (s, 10H, Cyclohexyl), 2.28 (s, 6H, C2-CH_3 and C6-CH_3), 4.90 (s, 1H, C4-H), 7.07-7.18 (m, 3H, phenyl) 7.20-7.27 (m, 2H, Phenyl), 8.63(s,1H, CO-NH), 8.94 (s, 2H, NH_2); MS (ESI): m/z: 502 $[M+1]^+$.

3.2. 2,2'-(1-cyclohexyl-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarbonyl) bis(hydrazinecarbothioamide) (6b)

IR (KBr, cm^{-1}): 3212 (-NH_2), 3175(NH), 3056 (C-H aromatic), 1720 (C=O, Carbonyl); ^{1}H NMR (DMSO – d6) δ ppm: 1.05-1.24 (m, 10H, Cyclohexyl), 2.32(s, 6H, C2-CH_3 and C6-CH_3), 5.01(s, 1H, C4-H), 9.07 (s, 2H, NH_2), 7.52-7.70 (m, 4H, Phenyl), 9.06 (s, 2H, NH_2); MS (ESI): m/z: 547 $[M+1]^+$.

3.3. 2,2'-(1-cyclohexyl-4-(4-methoxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5dicarbonyl) bis(hydrazinecarbothioamide). (6c)

IR (KBr, cm^{-1}): 3231 (-NH_2), 3198(NH), 3034 (C-H aromatic), 1698 (C=O, Carbonyl); ^{1}H NMR (DMSO – d6) δ ppm: 1.03-1.16 (m, 10H, Cyclohexyl), 2.25 (s, 6H, C2-CH_3 and C6-CH_3), 3.65 (s, 3H, OCH_3), 4.78(s, 1H, C4-H), 6.70-6.78(d, 2H, Aromatic), 7.01-7.08(d, 2H, Aromatic), 8.62(s, 1H, CO-NH), 8.85(s, 2H, NH_2); MS (ESI): m/z: 532 $[M+1]^+$.

3.4. 2,2'-(1-cyclohexyl-2,6-dimethyl-4-(p-tolyl)-1,4-dihydropyridine-3,5-dicarbonyl) bis(hydrazinecarbothioamide). (6d)

(KBr, cm^{-1}): 3224 (-NH_2), 3180 (NH), 3064 (C-H aromatic), 1710 (C=O, Carbonyl);^{1}H NMR (DMSO – d6) δ ppm: 0.99-1.27 (m, 10H, Cyclohexyl), 2.17 (s, 6H, C2-CH_3 and C6-CH_3), 3.17 (s, 3H, -CH_3), 4.89 (s, 1H, C4-H), 7.12-7.64(m, 5H, Aromatic), 8.62(s, 2H, NH_2); MS (ESI): m/z: 516 $[M+1]^+$.

3.5. 2,2'-(4-(4-chlorophenyl)-1-cyclohexyl-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarbonyl)bis(hydrazinecarbothioamide) (6e)

(KBr, cm^{-1}): 3220 (-NH_2), 3192 (NH), 3028 (C-H aromatic), 1698 (C=O, Carbonyl); ^{1}H NMR (DMSO – d6) δ

ppm: 1.01-1.28 (m, 10H, Cyclohexyl), 2.30(s, 6H, C2-CH_3 and C6-CH_3), 4.89(s, 1H, C4-H), 7.14-7.20(d, 2H, Aromatic), 7.24-7.30(s, 2H, Aromatic), 8.67(s, 1H, -CO-NH), 8.99(s, 1H, NH_2). MS (ESI): m/z: 537 $[M+1]^+$.

3.6. 2,2'-(1-benzyl-2,6-dimethyl-4-propyl-1,4-dihydropyridine-3,5-dicarbonyl) bis(hydrazinecarbothioamide) (6f)

(KBr, cm^{-1}): 3224 (-NH_2), 3180 (NH), 3064 (C-H aromatic), 1710 (C=O, Carbonyl); ^{1}H NMR (DMSO – d6) δ ppm: 0.91-1.39 (m,7H, propyl), 2.26(s, 6H, C2-CH_3 and C6-CH_3), 3.17 (s, 2H, CH_2-Benzyl), 3.67 (s, 3H, OCH_3), 4.80(s, 1H, C4-H), 6.70-6.81(d, 2H, Aromatic), 7.00-7.11(m, 3H, Aromatic), 8.64(s, 1H, -CO-NH), 8.76(s, 2H, NH_2). MS (ESI): m/z: 477 $[M+1]^+$.

3.7. 2,2'-(1-benzyl-4-butyl-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarbonyl) bis(hydrazinecarbothioamide) (6g)

(KBr, cm^{-1}): 3277 (-NH_2), 3175 (NH), 3029 (C-H aromatic), 1696 (C=O, Carbonyl); ^{1}H NMR (DMSO – d6) δ ppm: 0.93-1.47 (m,9H, butyl), 2.27(s, 6H, C2-CH_3 and C6-CH_3), 3.19(s, 2H, CH_2-Benzyl), 4.93(s, 1H, C4-H), 7.13-7.84 (m, 5H, Aromatic), 8.63(s, 1H, -CO-NH) 8.97(s, 2H, NH_2). MS (ESI): m/z: 491 $[M+1]^+$.

3.8. 2,2'-(1-benzyl-2,6-dimethyl-4-phenyl-1,4-dihydropyridine-3,5-dicarbonyl) bis(hydrazinecarbothioamide) (6h)

(KBr, cm^{-1}): 3226 (-NH_2), 3147 (NH), 3098 (C-H aromatic), 1698 (C=O, Carbonyl); ^{1}H NMR (DMSO – d6) δ ppm: 2.26 (s, 6H, C2-CH_3 and C6-CH_3), 3.15 (s, 2H, CH_2-Benzyl), 4.89 (s, 1H, C4-H), 7.01-7.30 (m, 7H, aromatic) 7.20-7.27 (m, 3H, aromatic), 8.63 (s, 1H, CO-NH), 8.91 (s, 2H, NH_2). MS (ESI): m/z: 510 $[M+1]^+$.

3.9. 2,2'-(1-benzyl-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarbonyl) bis(hydrazinecarbothioamide) (6i)

(KBr, cm^{-1}): 3220 (-NH_2), 3192(NH), 3028 (C-H aromatic), 1698 (C=O, Carbonyl); ^{1}H NMR (DMSO – d6) δ ppm: 2.27 (s, 6H, C2-CH_3 and C6-CH_3), 3.18 (s, 2H, CH_2-Benzyl), 4.95 (s, 1H, C4-H), 6.69-6.81 (d, 2H,) 7.13-7.48 (m, 5H, aromatic), 4.48-7.53(d, 1H,), 7.57-7.62 (d,1H,), 8.65 (s, 1H, CO-NH), 8.99 (s, 2H, NH_2). MS (ESI): m/z: 555 $[M+1]^+$.

3.10. 2,2'-(1-benzyl-4-(4-methoxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarbonyl) bis(hydrazinecarbothioamide) (6j)

(KBr, cm^{-1}): 3243 (-NH_2), 3194 (NH), 3088 (C-H aromatic), 1705 (C=O, Carbonyl); ^{1}H NMR (DMSO – d6) δ ppm: 2.24 (s, 6H, C2-CH_3 and C6-CH_3),3.17 (s, 2H, CH_2-Benzyl), 3.66 (s, 3H, OCH_3), 4.80 (s, 1H, C4-H), 6.69-6.81 (d, 2H,) 7.00-7.11 (d, 2H,), 7.20-7.64 (m, 5H, Benzyl), 8.64 (s, 1H, CO-NH), 8.77 (s, 2H, NH_2). MS (ESI): m/z: 540 $[M+1]^+$.

3.11. 2,2'-(1-benzyl-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarbonyl)

bis(hydrazinecarbothioamide) (6i)

(KBr, cm^{-1}): 3220 ($-NH_2$), 3192(NH), 3028 (C-H aromatic), 1698 (C=O, Carbonyl); ^1H NMR (DMSO – d6) δ ppm: 2.27 (s, 6H, C2-CH_3 and C6-CH_3), 3.18 (s, 2H, CH_2-Benzyl), 4.95 (s, 1H, C4-H), 6.69-6.81 (d, 2H,) 7.13-7.48 (m, 5H, aromatic), 4.48-7.53(d, 1H,), 7.57-7.62 (d,1H,), 8.65 (s, 1H, CO-NH), 8.99 (s, 2H, NH_2). MS (ESI): m/z: 555 $[M+1]^+$.

3.12. 2,2'-(1-benzyl-4-(4-methoxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarbonyl) bis(hydrazinecarbothioamide) (6j)

(KBr, cm^{-1}): 3243 ($-NH_2$), 3194 (NH), 3088 (C-H aromatic), 1705 (C=O, Carbonyl); ^1H NMR (DMSO – d6) δ ppm: 2.24 (s, 6H, C2-CH_3 and C6-CH_3),3.17 (s, 2H, CH_2-Benzyl), 3.66 (s, 3H, OCH_3), 4.80 (s, 1H, C4-H), 6.69-6.81 (d, 2H,) 7.00-7.11 (d, 2H,), 7.20-7.64 (m, 5H, Benzyl), 8.64 (s, 1H, CO-NH), 8.77 (s, 2H, NH_2). MS (ESI): m/z: 540 $[M+1]^+$.

3.13. 2,2'-(1-benzyl-2,6-dimethyl-4-(p-tolyl)-1,4-dihydropyridine-3,5-dicarbonyl) bis(hydrazinecarbothioamide) (6k)

(KBr, cm^{-1}): 3251 ($-NH_2$), 3192 (NH), 3056 (C-H aromatic), 1727 (C=O, Carbonyl); ^1H NMR (DMSO – d6) δ ppm: 2.28 (s, 6H, C2-CH_3 and C6-CH_3), 3.21(s, 2H, CH_2-Benzyl), 4.94 (s, 1H, C4-H), 7.11-7.19 (d, 4H,), 7.31-7.64 (m, 5H, Benzyl), 8.63 (s, 1H, CO-NH), 8.81 (s, 2H, NH_2). MS (ESI): m/z: 525 $[M+1]^+$.

3.14. 2,2'-(1-benzyl-4-(4-chlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarbonyl) bis(hydrazinecarbothioamide) (6l)

(KBr, cm^{-1}): 3259 ($-NH_2$), 3136(NH), 3059 (C-H aromatic), 1693 (C=O, Carbonyl); ^1H NMR (DMSO – d6) δ ppm: 2.26 (s, 6H, C2-CH_3 and C6-CH_3), 3.17 (s, 2H, CH_2-Benzyl), 4.84 (s, 1H, C4-H), 7.08-7.17 (d, 2H,) 7.19-7.28 (d, 2H,), 7.31-7.64 (m, 5H, Benzyl), 8.63 (s, 1H, CO-NH), 8.94 (s, 2H, NH_2). MS (ESI): m/z: 544 $[M+1]^+$.

4. Results and Discussion

A series of 2,2'-[(4-substituted)1-cyclohexyl/benzyl-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarbonyl)] bis(hydrazinecarbothioamides) were synthesized by the reaction of 4-Alkyl/Aryl-1-substituted-3,5-bis(carboethoxy)-2,6-dimethyl-1,4-dihydropyridines with thiosemicarbazide. The physical data of the compounds were presented in Table-1. The IR spectrum (KBr pellet) of 6a-6l showed absorption band in between 3220-3277cm-1 (NH_2). The stretching band was noticed at ~ 3136 cm^{-1} (-NH) while carbonyl group (C=O) stretching peak was found in between 1693-1761 cm-1. ^1H NMR spectrum of compounds 6a-6l showed peak in the range of δ 8.62-9.07 due to NH_2. The singlet peaks at ~ 5.1 and ~ 8.63 were assigned to 4-CH of DHP and CO-NH respectively. The mass spectrum of the compounds showed the molecular ion peak which confirms the molecular mass of the compounds.

Among all the compounds tested, compound 6l with N-benzyl and 4-(4-chlorophenyl) substitution exhibited the

highest potency against MCF-7 and HeLa cell lines with IC_{50} values of 56.8 and 66.5 µM respectively. Whereas compound 6j showed highest potency against Hep G2 cell lines with IC50 of 68.3 µM. Replacement of 4-(4-chlorophenyl) group with N-butyl and N-propyl (6f and 6g) resulted in significant decrease in potency against MCF-7 and HeLa cell lines (IC50 values of 6f: > 100 µM against MCF-7 and HeLa) and (IC50 values of 6g 86.2 and > 100 µM against MCF-7 and HeLa respectively). 1-Benzyl group appears to be contributing to the activity against all the three cell lines, as its replacement with cyclohexyl group resulted in significant decrease in potency. For example compound 6e with 4-(4-chlorophenyl) and 1-cyclohexyl substitution exhibited IC50 of 81.7 µM, compared to 56.8 µM shown by its 1-benzyl analog (6l) against MCF-7 cell lines. Among the 1-cyclohexyl substituted compounds (6a-6e), the compound 6e with 4-(4-chlorophenyl) substitution showed highest potency against all three cell lines (MCF-7, HeLa and HepG2) with IC50 values 81.7, 78.1 and 79.5 µM respectively.

Table 1: Physical properties of new 1,4-dihydropyridine derivatives (6a-6l)

COMPOUND	**-R**	**R_1**	**MOLECULAR WEIGHT (gm/mole)**	**MELTING POINT (^{O}C)**	**% YIELD**
6a	-C_6H_5	Cyclohexyl	501	168-169	59.3
6b	-C6H4. NO2 -3	Cyclohexyl	546	211-214	61.6
6c	-C6H4. OCH3 -4	Cyclohexyl	531	201-204	63.2
6d	-C6H4. CH3 -4	Cyclohexyl	515	209-211	49.6
6e	-C6H4. Cl -4	Cyclohexyl	536	214-216	57.3
6f	-n-Pr	Benzyl	476	201-204	63.7
6g	n-Bu	Benzyl	490	195-197	52.8
6h	-C_6H_5	Benzyl	509	211-213	60.4
6i	-C6H4. NO2 -3	Benzyl	554	252-255	46.8
6j	-C6H4. OCH3 -4	Benzyl	539	240-242	55.7
6k	-C6H4. CH3 -4	Benzyl	523	218-221	61.6
6l	-C6H4. Cl -4	Benzyl	543	231-233	59.5

5. Conclusion

In conclusion, all the synthesized new 1,4-dihydropyridine derivatives (6a-6l) were found to be less potent than the standard cisplatin against the three cell lines tested. However, among them compounds 6j and 6l showed better activity compare to the other derivatives.

Table 2: Anticancer activity of new 1,4-dihydropyridine derivatives (6a-6l)

COMPOUND	-R	R_1	IC_{50} (µM)		
			MCF-7	HeLa	Hep G2
6a	$-C_6H_5$	Cyclohexyl	>100	87.8	96.4
6b	-C6H4. NO2 -3	Cyclohexyl	88.2	>100	>100
6c	-C6H4. OCH3 -4	Cyclohexyl	82.6	91.4	92.8
6d	-C6H4. CH3 -4	Cyclohexyl	93.5	86.3	>100
6e	-C6H4. Cl -4	Cyclohexyl	81.7	78.1	79.5
6f	-n-Pr	Benzyl	>100	>100	>100
6g	n-Bu	Benzyl	86.2	>100	96.4
6h	$-C_6H_5$	Benzyl	84.5	>100	85.3
6i	-C6H4. NO2 -3	Benzyl	92.7	>100	95.8
6j	-C6H4. OCH3 -4	Benzyl	67.3	72.4	68.3
6k	-C6H4. CH3 -4	Benzyl	79.6	>100	75.4
6l	-C6H4. Cl -4	Benzyl	56.8	66.5	73.8
Cisplatin			12.7	9.3	13.8

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