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SYNTHESIS, CHARACTERIZATION ANDINVITRO CYTOTOXIC EVALUATION OF NOVEL AMIDE DERIVATIVES OF 5-[2-(4-METHOXYPHENYL)PYRIDIN-3-YL]-1, 3, 4-THIADIAZOL-2-AMINE.

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ABSTRACT

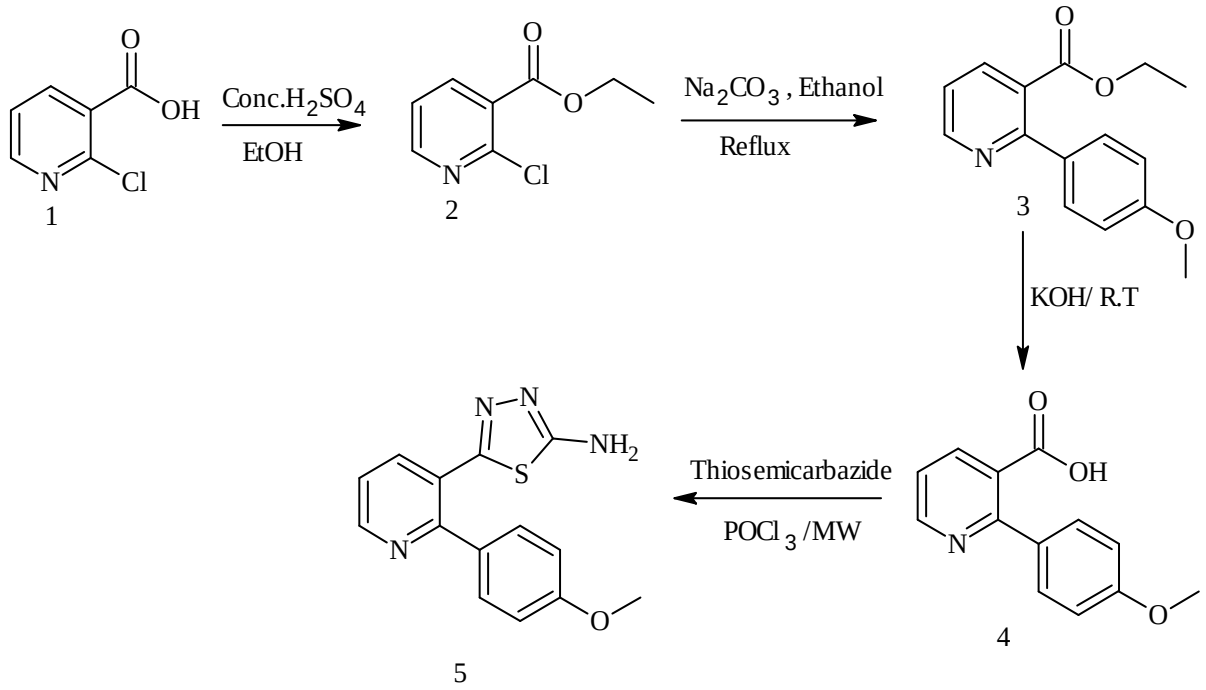
In this research we have synthesized five novel amide derivatives of 5-[2-(4-methoxyphenyl) pyridin-3-yl]-1, 3, 4-thiadiazol-2-amine and evaluated their cytotoxicity by MTT assay. Three cell lines were used for the evaluation HeLa, HepG2 and PANC-1. All the synthesized compounds were characterized by LCMS, IR, 1H and 13C (proton and Carbon 13) spectroscopies and elemental analysis. These compounds were evaluated for invitro anticancer activity on three different human leukemic cell lines, namely HeLa, $HepG_2$ and $PANC\text{-}_1$.In total five compounds were synthesized and studied for their MTT assay. Among five synthesized novel compounds, the compound 2-chloro-N-{5-[2-(4-methoxyphenyl)pyridin-3-yl]-1, 3, 4-thiadiazol-2-yl} pyridine-3-carboxamide6d is highly cytotoxic on HeLa and PANC-1 cell lines having IC_{50} of 2.8µM and 1.8 µM respectively. Rest all the compound showed less cytotoxicity on all the three cell lines as compared with the standard 5-FU1.

KEY WORDS: HeLa, 1, 3, 4-thidiazoles, Thiosemicarbazide, Anticancer, MTT assay.

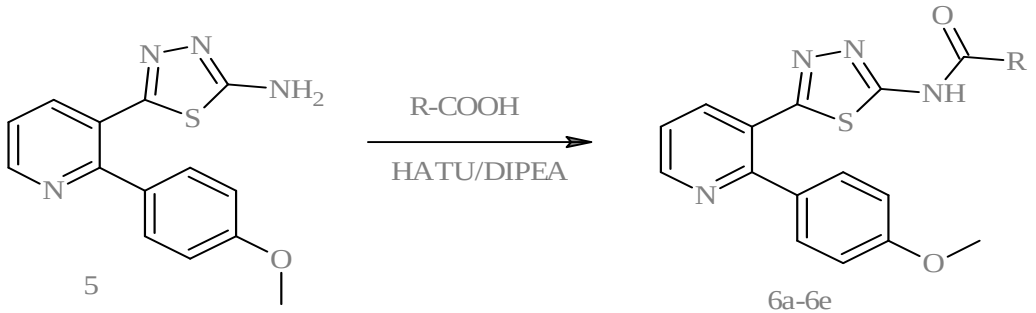
INTRODUCTION

Author has synthesized the novel amide derivatives of 5-[2-(4- methoxyphenyl) pyridin-3-yl]-1, 3, 4-thiadiazol-2-amine and screened these compounds forcytotoxicity[1] on three different human leukemic cell lines. Synthetic chemistry was started with 2-chloro nicotinic

acid which is converted into ethyl ester and subsequently synthesized the carbohydrazide **4**. Thus obtained carbohydrazide was cyclized using phosphorous oxy chloride and thiosemicarbazide in order to get the key intermediate.This kind of novel ring systems not yet studied but few of the derivatives of pyridine containing 1, 3, 4-thiadiazolemoiety have been reported for their potent activity towards anticancer [1] anti-tubercular [3] anti-inflammatory[4, 5, 6] anti-bacterial [7, 8] and kinase inhibition[9, 10] properties. In this connection the author envisaged that by attaching 4-methoxy phenyl group at the second position of the pyridine and constructed 1, 3, 4-thiadiazole moiety.This constitutes the basic ring system and may enhance the Log-P values and thus increasing the potency. In order to validate this hypothesis the author has synthesized five novel amide derivatives of 1, 3, 4-thiadiazole [13, 14] compounds and tested their invitro cytotoxicity against cancer cell lines. The study revealed that the different 1, 3, 4-thiadiazole derivatives possesses excellent anticancer activity[15].In this synthesis compounds **6d**has showed good antiproliferative[15] activity on HeLaand PANC-1 cell lines having IC50 of 4.6µM and 2.2µMrespectively.



Scheme 1: Synthesis of5-[2-(4-methoxyphenyl) pyridin-3-yl]-1, 3, 4-thiadiazol-2-amine(Intermediate)



5-[2-(4-methoxyphenyl)pyridin-3-yl]-1,3, 4-thiadiazol-2-amine

R-Substituted aromatic acids

EXPERIMENTAL

MATERIALS AND METHODS

All reagents, chemicals and solvents were purchased from S-d fine and Spectrochem ltd.Bengaluru.India.^{1}H NMR and ^{13}C NMR were recorded by Brucker 400 MHz spectrophotometer. Melting points are determined using Buchi melting point 545.Mass spectra were recorded by Agilent 1200 series.TLC was done on F254 grade silica 60 from Merck.IR spectra was recorded by FTIR (1800S) series.

Synthesis

Step 1. Synthesis of Ethyl 2-Chloropyridine-3-Carboxylate 2

The 2-chloronicotinic acid **1** (10g, 0.0636mol) was taken in a 1L single necked round bottom flask, 250mL of ethanol and concentrated H_2SO_4(3-5 drops) were added, reaction mixture was refluxed at 85°C for 10 hr. TLC(Thin layer chromatography) was monitored to check the completion of the reaction. Solvent was evaporated and the residue was neutralized with 10% $NaHCO_3$solution. Aqueous was extracted with ethyl acetate (50x2mL), washed with brine (25mL) and dried over Na_2SO_4, evaporated. The obtained pale yellow oil was recrystallized form ethanol-water as yellow needles. Yield 8.5g, MS-[M+H]- 187; HPLC purity = 95.7%; TLC-ethyl acetate: hexane (1:9); IR(KBr), νmax/cm^{-1}: 984, 1189, 2845, 3106 ; ^{1}HNMR ($CDCl_3$, 400MHz) : δ 1.19(t, 3H), 3.79(q, 2H), 7.46(t, 1H, J 13.4Hz), 8.45(dd, 1H, J 8.5Hz), 8.88(d, 1H, J 7.8 Hz).

Step 2: Synthesis of Ethyl 2-(4-methoxy phenyl) Pyridine-3-Carboxylate 3: Ethyl2-chloropyridine-3-carboxylate(8.5g, 0.0457mol),Na_2CO_3(19.37g, 0.182mol),4-methoxy phenylboronicacid (8.335g, 0.0448mol),tetrakis (triphenyl phosphine)palladium (0)(0.263g,304.8mol) were refluxed in 150mL of ethanol for 11h.TLC was monitored to check the completion of the reaction, after completion, the solvent was evaporated,aqueous

wasextracted with ethyl acetate (35x4mL), washed with brine (25mL) and dried over Na_2SO_4. Ethyl acetate was evaporated to yield brown oil. The crude product was purified by column chromatography using silica gel(100 to 200mesh), gradient (0-25%) ethyl acetate in hexane as the eluent. Yield 4.6g,off white coloured solid ;ms(ESI) m/z: [M+H]-258; m.p-145-146^{0}C; IR(KBr),νmax/cm^{-1} : 1170, 2985,3166; ^{1}H-NMR($CDCl_3$, 400 MHz) : δ 0.93(t,2H), 2.64(s, 3H), 3.67(q,3H), 7.36(dd,J 7.8 Hz, 2H), 7.58(q,2H), 8.79(m,J 13.2Hz, 1H), 9.44(q,2H).

Step 3: Synthesis of2-(4-methoxyphenyl)pyridine-3-carboxylic acid: (4)

The Ethyl 2-(4-methoxy phenyl) Pyridine-3-Carboxylate(4.6g, 0.02008mol) was taken in a 250ml RB flask containing 150 mL of methanol. To this reaction mixture KOH (3.714g, 0.1004mol) was added and kept for stirring at R.T overnight. After completion, the solvent was removed under reduced pressure and added with 150 mL of ice cold water. On acidification with concentrated HCl (P^H 3- 4) precipitates that are formed were filtered, washed with cold water and dried over vacuum. Yield = 4.2 g, MS: [M+] 229, [M-H] 228; Pale brown solid; M.P- 145-148^{0}C; IR(KBr),νmax/cm^{-1}- 1235, 2353, 2450, 3117cm^{-1};^{1}H-NMR-δ 2.6(s, 3H), 7.23-7.15(dd,2H), 7.64(dd,2H), 7.85-7.65(t,2H), 9.3(m,1H), 11.1(bs,1H).

Step 4; Synthesis of 5-[2-(4-methoxyphenyl)pyridin-3-yl]-1,3,4-thiadiazol-2-amine(5):

The 2-(4-methoxyphenyl) pyridine-3-carboxylic acid (4.2g, 0.0147mol) and thiosemicarbazide (2.691g, 0.0295mol) were taken in a 250mL beaker to this phosphorous oxychloride(25mL) was added and made paste. This paste was irradiated with microwave for a period of 15 minutes (30sec/interval).The bright yellow solid was obtained after the basification with ammonia. Solids that are separatedwasfiltered washed with water and dried over vacuum. The yellow coloured solid; Yield = 2.6g; m.p = 166-168^{0}C (decomposes); MS: [M+] 284, [M-H]-283; (KBr), νmax/cm^{-1}- 1210, 1456, 2925,3126,3428,cm^{-1}; ^{1}H NMR-δ 2.62(s, 3H), 5.23-5.13(bs, 2H), 7.27(dd,2H), 7.67(t,1H), 7.75(dd,2H), 8.1(d, 2H).

General Procedure for the Synthesis of Amide Derivatives of 5-[2-(4-methoxyphenyl) pyridin-3-yl]-1, 3, 4-thiadiazol-2-amine6a-6e

All the reactions were carried out under nitrogen atmosphere. In a 100ml round bottom containing 200mg of 5-[2-(4-methoxyphenyl) pyridin-3-yl]-1, 3, 4-thiadiazol-2-amine(Scheme 2, compound 5), different substituted aromatic acids (1.1.eqivalent), HATU (1 equivalent) and di isopropyl ethyl amine (2 equivalent) were added under stirring. DCM (Dichloromethane) was added and the RM was stirred overnight. After the completion of the reaction, solvent was removed completely, residue was added with crushed ice and solids that

are separated was filtered, washed with saturated solution of $NaHCO_3$ and dried. The crude product was purified by column chromatography using silica gel 100-200mesh and gradient (0-80%) ethyl acetate in hexane as eluent.

Analytical data of the final novel amide derivatives of 5-[2-(4-methoxyphenyl) pyridin-3-yl]-1, 3, 4-thiadiazol-2-amine6a-6e

2-Fluoro-N-{5-[2-(4-methoxy-phenyl)-pyridin-3-yl]-[1, 3, 4] thiadiazol-2-yl}-benzamide(6a): R = 2-Fluoro benzoic acid

white coloured solid; yield 52.8% ; m.p -175-178^{0}C; IR (KBr), ν_{max}/cm^{-1} : 1133, 2765, 2945, 3320 ; ^{1}H-NMR($CDCl_3$, 400MHz): δ 2.32(s, 3H, OCH_3), 7.2(dd, J 8.4Hz, 2H), 7.4(m, 4H), 7.65(m, 3H), 7.8(dd, J 7.2Hz, 2H), 9.1(dd, J 12.4Hz, 2H), 10.02(bs, 1H,NH); ^{13}C NMR($CDCl_3$, 100MHz): 65, 114, 124, 128, 130, 134, 135, 137, 144.5, 150, 155, 159, 163, 170 ; molecular formula $C_{21}H_{15}FN_4O_2S$;ms: (ESI) m/z:[M+H]- 407; HPLC 94.4% ;anal. Calculated for $C_{21}H_{15}FN_4O_2S$; C, 62.06; H, 3.72; F, 4.67; N, 13.79; O, 7.87; S, 7.89; Found C, 62.07; H, 3.73; F, 4.68; N, 13.80; O, 7.88; S, 7.90.

2,5-Dimethoxy-N-{5-[2-(4-methoxy-phenyl)-pyridin-3-yl]-[1,3,4]thiadiazol-2-yl}-benzamide (6b): R = 2,5-Dimethoxy benzoic acid.

Off white coloured solid; yield 57% ; m.p -135-136^{0}C; IR (KBr), ν_{max}/cm^{-1} : 1255, 2865, 2985, 3318, 3386 ;^{1}H-NMR ($CDCl_3$, 400MHz): δ2.3(s, 9H), 7.14(m, 3H), 7.2(dd, J 8.3Hz, 2H), 7.7(dd, J 8.5Hz, 2H), 8.2(dd, 2H), 9.2(dd, J 13.4Hz, 1H), 10.05(bs, 1H, NH); ^{13}C NMR($CDCl_3$, 100MHz): 65, 114.5, 115, 120, 124, 128, 134, 135, 137, 144.5, 150, 151, 152, 155, 159, 163, 170; molecular formula $C_{23}H_{20}N_4O_4S$; ms: (ESI) m/z:[M+H]-449; HPLC 94.7% ; anal. Calculated for $C_{23}H_{20}N_4O_4S$; C, 61.59; H, 4.49; N, 12.49; O, 14.27; S, 7.15 Found C, 61.60; H, 4.50; N, 12.50; O, 14.28; S, 7.16.

4-Methoxy-N-{5-[2-(4-methoxy-phenyl)-pyridin-3-yl]-[1, 3, 4] thiadiazol-2-yl}-benzamide(6c): R = 4-Methoxy benzoic acid.

Off white coloured solid; yield 57%; IR (KBr), ν_{max}/cm^{-1} : 1225, 2798, 2825, 2965, 3356, 3350; ^{1}H-NMR ($CDCl_3$, 400MHz):δ2.32(s, 6H),7.22(dd, J 13.4Hz, 2H), 7.6(m, 3H), 7.75(dd, J 8.4Hz, 2H), 8.2(dd, 2H), 9.1 (dd, J 6.8Hz, 2H), 10.03(bs, 1H,NH); ^{13}C NMR($CDCl_3$, 100MHz): 65, 113, 114.5, 124, 126, 128.5, 129, 134, 135, 137, 150, 155, 158, 164, 166, 170;molecular formula $C_{22}H_{18}N_4O_3S$;ms: (ESI) m/z:[M+H]- 419; HPLC 95.2% ; anal. Calculated for $C_{22}H_{18}N_4O_3S$; C, 63.14; H, 4.34; N, 13.39; O, 11.47; S, 7.66; Found C, 63.15; H, 4.36; N, 13.40; O, 11.48; S, 7.67.

2-Chloro-N-{5-[2-(4-methoxy-phenyl)-pyridin-3-yl]-[1, 3, 4] thiadiazol-2-yl}-nicotinamide (6d): R = 2-Chloronicotinic acid

white coloured solid; yield 66%; m.p- 172-176°C ; IR (KBr), ν_{max}/cm^{-1} : 1285, 2886, 2890, 2935, 3259, 3396 ; ^{1}H-NMR($CDCl_3$, 400MHz): δ2.3(s, 3H), 7.2(dd, 2H), 7.6(m, 3H), 7.75(dd, 2H), 8.1(m, 3H), 10.03(bs, 1H,NH); ^{13}C NMR($CDCl_3$, 100MHz): 65, 114.5, 119, 121, 134, 135, 137, 150, 155, 156, 159, 163, 170; molecular formula $C_{20}H_{14}ClN_5O_2S$ms: (ESI) m/z:[M+H]- 424; HPLC 96% ;anal. Calculated for $C_{20}H_{14}ClN_5O_2S$; C, 56.67; H, 3.33; Cl, 8.36; N, 16.52; O, 7.55; S, 7.56; Found C, 56.68; H, 3.34; Cl, 8.37; N, 16.53; O, 7.56; S, 7.57.

4'-Fluoro-biphenyl-2-carboxylic acid {5-[2-(4-methoxy-phenyl)-pyridin-3-yl]-[1, 3, 4] thiadiazol-2-yl}-amide (6e): R = 4-Fluorobiphenyl-2-carboxylic acid.

Pale yellow coloured solid; yield 57% ; m.p: 189-191^{0}C IR (KBr), ν_{max}/cm^{-1} : 1285,2854, 2945, 3256, 3326 ; ^{1}H-NMR($CDCl_3$, 400MHz): δ 2.05 (s, 3H, O-CH_3), 7.32(dd, J 12.4, 2H), 7.45(dd, 2H), 7.8(m, 3H), 8.12(m, 3H), 8.3(dd, 2H), 9.1(dd, J 7.8Hz, 2H), 10.06(bs, 1H,NH); ^{13}C NMR($CDCl_3$, 100MHz): 65, 114.5, 116, 122, 124, 129, 134, 135, 136, 137, 150, 154, 156, 164, 170; molecular formula $C_{27}H_{19}FN_4O_2S$; ms: (ESI) m/z:[M+H]- 483; HPLC 96% ; anal. Calculated for $C_{27}H_{19}FN_4O_2S$; C, 67.21; H, 3.97; F, 3.94; N, 11.61; O, 6.63; S, 6.65; Found C, 67.22; H, 3.98; F, 3.95; N, 11.63; O, 6.64; S, 6.66.

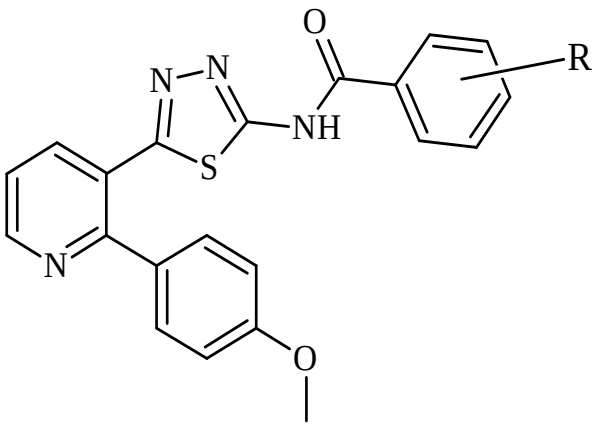


Table 1: **IC_{50} values of the novel amidederivatives of 5-[2-(4-methoxyphenyl) pyridin-3-yl]-1, 3, 4-thiadiazol-2-amine 6a-6e:**

Compounds 6a-6e	**IC_{50} values of the 1,3,4-thiadiazole amides In µM**		
HeLaHepG$_2$	PANC-$_1$		
6a	34.4	65.6	126.7
6b	124.9	56.7	27.8
6c	54.6	78.6	34.3
6d	2.8	67.5	1.8
6e	112.3	76.7	45.5
5-U^2	7.8	6.9	8.2

IC_{50}- Is the concentration that induces 50% of the growth inhibition as compared to untreated cells. 5-FU- 5-Fluoro uracil, standard used in the experiment.

Cytotoxic Evaluation

Cell Lines fixation and Culture Conditions

Theinvitro anti-proliferative study was carried out on three human carcinoma cell lines namely HeLa, HepG2 and PANC-1.All the cell lines were grown in DMEM-HG supplemented with 10% heat-inactivated FBS, 2% Penicillin-Streptomycin and 2.5 µg/mL Amphotericin-B solutions (All from HI Media Labs, Mumbai, India).Cell lines were incubated at 37°C in a humidified atmosphere of 95% air, 5% CO_2. Following 24-48 hr.of incubation period, the adherent cells were detached using Trypsin-EDTA solution (HI Media Labs, Mumbai, India). Cell count was done using the Luna automated cell counter (Logos Bio systems, India) based on trypan blue dye exclusion method. Cytotoxicity of the novel amidederivatives of 1, 3, 4-thiadiazoles have been determined using MTT 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay.

Invitro Cell Viability Assay (MTT Assay)

200µL cell suspension was seeded in 96-well micro plates (Corning®, USA**)** at a density of 25,000 cells/well and incubated for 24hrs, all cells were seeded in duplicates with novel compounds **6a-6e.**Having range of concentrations from 50µM-500µM, incubated in a CO_2 incubator at 37°C. Treated cells were thereafter incubated with 10% MTT (5mg/ml; HI Media Labs, Mumbai, India) for 3 h.The culture medium was then aspirated and 200µL dimethyl sulfoxide (DMSO; Sigma-Aldrich, India) was added. 5-fluorouracil was used as control. Cell viability was determined by measuring the absorbance on a micro plate reader (SPECTRO STAR NANO, BMG LABTECH, Germany) at 570nm. Cell viability was

calculated as a percentage of viable cells at different test concentrations relative to the control (5-FU) cells [% cell viability = (A_{570} of treated cells / A_{570} of control cells) ×100%].

RESULTS AND DISCUSSIONS

Chemistry (Figure 1&2): The synthetic chemistry of the novel amide derivatives of 1, 3, 4-thiadiazole**6a-6e** compounds started with the synthesis of key intermediate5-[2-(4-methoxyphenyl) pyridin-3-yl]-1, 3, 4-thiadiazol-2-amine[16, 17, 18]**5**. This intermediate was obtained by reacting compound **4** with phosphorous oxy chloride and thiosemicarbazide.The compound **4** was obtained by Suzuki reaction [18, 19]to the compound **3** in presence of tetrakis (triphenyl phosphine) palladium (0). The obtained compound **5** (1, 3, 4-thiadiazole amine) was treated with various acids to obtain thefinal amide compounds **6a-6e.**Author envisaged that by introducing 4-methoxyphenyl boronic acid group at the second position of the pyridine ring may increase the the Log-P and TPSA values of 1, 3, 4-thiadizoles and thus increasing the more bioavailability of the compounds.

SAR: Structural Activity Relationship

Studies related to SAR of these 1, 3, 4-thiadiazoles[19]amide derivativesshowed increase in potency towards antiproliferative activity. The 4-methoxy phenyl group substituted pyridine ring increases the water solubility and thereby more bio available molecules.By introducing the 4-methoxy phenyl group at the second position of the pyridine enhances further the Log-P values as well as increases the TPSA of the molecules. Author envisaged that by coupling different acids in presence of HATU to obtain the corresponding amide compounds may show the good anticancer properties.

Biology

The obtained series of novel 1, 3, 4-thiadiazole derivatives **6a-6e** have been screened for cytotoxicity[19, 20] on three different human leukemic cell lines to obtain the IC_{50} of the molecules. The cancer cell lines used was HeLa, HepG2 and PANC-1.The MTT assay of the novel 1, 3, 4-thiadaizoles[20] have been screened for these cell lines and obtained the interesting data (Table 1).Compound **6d** showed greater cytotoxicity on HeLaand PANC-1 cell lines having IC_{50} of 2.8µM and 1.8 µM respectively. Rest all the compounds showed moderate cytotoxicity as in the (Table 1).

CONCLUSIONS

In this research author has synthesized five novel derivatives of 1, 3, 4-thiadiazole and screened for MTT assay.Compound **6d** showed good antiproliferative activity on HeLa and PANC-1 cell lines having IC_{50} 2.8µM and 1.8µM of respectively.Rest all the compounds showed moderate to low cytotoxicity on all the three cell lines as compared with the standard 5-FU.

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REFERENCES

1. Rajyalakshmi G, Rama N, Sarangapani M. Synthesis, characterization and anticancer activity of certain 3-{4-(5-mercapto-1, 3, 4-oxadiazole-2-yl)phenylimino}indolin-2-one derivatives. Saudi Pharmaceutical Journal, 2011; 19:153–158.
2. Anees P, JavedAS.Various pharmacological aspects of 2, 5-disubstituted 1, 3, 4-oxadiazole derivatives A Review. Res. J. Chem. Sci., 2013;3 (12): 79-89.
3. Joshi SD, Vagdevi HM, Vaidya VP, Gadaginamath GS. Synthesis of new 4-pyrrol-1-yl benzoic acid hydrazide analogs and some derived oxadiazole, triazole and pyrrole ring systems: A novel class of potential antibacterial and antitubercular Agents. Eur. J. Med. Chem., 2008; 43:1989-1996
4. Arvind KS, Lohani M, Parthsarthy M. Synthesis, characterization and anti-Inflammatory activity of some 1, 3,4 -oxadiazole derivatives. IJPR, 2013; 12 (3): 319–323.
5. Bachwani M, Sharma V, Kumar R.Biological activities of 1, 3, 4-oxadiazole A review.Int. Res. J. Pharm., 2011;2 (12): 84-89
6. Balasaheb YM, Vidyadhara S. Synthesis and screening of anti-Inflammatory activity of benzofuran derivatives bearing oxadiazole.Orient. J. Chem., 2011;27(3): 1227-1231.
7. Mohamed A A. Synthesis and antimicrobial activity of new 5-(2-thienyl)-1, 2, 4-triazoles and 5-(2-thienyl)-1, 3,4-oxadiazoles and related derivatives. Molecules,2010; 15: 502-514.
8. Aziz UR, Siddiqui, SZ, Abbasi MA, Abbas N, Khan KM, Shahid M, Mahmood Y, Akhtar MN, Lajis NH. Synthesis, antibacterial screening and hemolytic activity of s-substitutedderivatives of 5-benzyl-1, 3, 4-oxadiazole-2-thiol.Int. J. Pharm. & Pharma. Sci., 2012; 4 (2): 676-680.

9. Zhang S,Luo Y, He, LQ, Liu ZJ, Jiang AQ, Yang YH, Zhu HL. Synthesis, biological evaluation, and molecular docking studies of novel 1, 3, 4-oxadiazole derivatives possessing benzotriazole moiety as FAK inhibitors with anticancer activity. Bioorg.Med. Chem., 2013; 21 (13):3723-9
10. Saitoh M, Kunitomo J, Kimura E, Iwashita H, Uno Y, Onishi T, Uchiyama N, Kawamoto T, Tanaka T, Mol CD, Dougan DR, Textor GP, Snell GP, Takizawa M, Itoh F, Kori M. 2-{3-[4-(Alkylsulfinyl) phenyl]-1-benzofuran-5-yl}-5-methyl-1, 3, 4-oxadiazole derivatives as novel inhibitors of glycogen synthase kinase-3beta with good brain permeability.J. Med. Chem., 2009; 52 (20):6270-6286.
11 Alok,P, Rajavel, R, Sandeep, C, Deepak, D. Synthesis of Schiff bases of 2-amino-5-aryl-1, 3, 4-thiadiazole and its analgesic, anti-inflammatory and anti-bacterial activity.E-Journal of Chemistry, 2012; 9 (4): 2524-2531.
13. Xian H Y, Qing W, Ting T Z, Jian S, Xi Li, Man X, Xiang L, Hai-Liang Z. Synthesis, biological evaluation, and molecular docking studies of cinnamic acyl 1,3,4-thiadiazole amide derivatives as novel antitubulin agents.Bioorg. Med. Chem.,2012; 20(3):1181-7
14. Kratika S, Suresh P and Sarita S. Studies On Nitrogen And Sulphur Containing Heterocyclic Compound: 1,3,4-thiadiazole. Asian J. Biomed.Pharma. Sci., 2013; 3(21): 6-23
15. Huaiwei D, Zhe C, Cunlong Z, Tian X, Yini W, Hongrui S,Yuyang J,Yuzong C, Yongnan X, Chunyan T. Synthesis and Cytotoxic Activity of Some Novel N-Pyridinyl-2-(6-phenylimidazo [2, 1-b] thiazol-3-yl)acetamide Derivatives.Molecules,2012;17: 4703-4716.
16. Mullick P, Khan SA, Verma S, Alam O. Thiadiazole Derivatives As Potential Anticonvulsant Agents, Bulletin Of The Korean Chemical Society, 2011; 32(3): 1011-1016.
17. Rajesh S, Jitendra S, Subhash CC. 2-Amino-5-Sulfanyl-1,3,4-Thidiazoles: A New Series Of Selective Cyclooxygenase-2 InhibitorsActa Pharm., 2008; 58: 317–326. 10.2478/V10007-008-0011-6.
18. Suzuki N, TamotsuaibaraShunzo .Eur. Pat. Appl., 2008, Ep 159707 A2 19851030.
19. Suzuki NM, Tamotsuaibara, Shunzokanno, Hideyuki T, Hideo Tsubokawa, Masao, R, Yuichi T, Wataruisoda, Sumiro. Synthesis And Antiallergy Activity Of [1,3,4]Thiadiazolo[3,2-A]-1,2,3-Triazolo[4,5-D]Pyrimidin-9(3h)-One Derivatives, Chem.Pharm. Bull., 1992; 40(2): 357-363.

20. Rajasekaran S, Gopal K R, Sanjay P, Gurpreet K V, Gurpreet S S. Synthesis and invitrostudy of biological activity of 2,3-substitutedquinazolin-4(3H)-ones. J. Chem. Pharm. Res.,2010; 2 (2): 462-468.

21 .Krunal VJ, Nikhil MP, Bhaskar MR. Synthesis and antibacterial activities of N-chloro aryl acetamidesubstituted thaizole and 2, 4-thazolidinedione derivatives. Arch. Appl. Sci. Res., 2011; 3 (5):540-548.