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Synthesis, Characterization and Cytotoxic Evaluation of Novel Schiff Base Derivatives of 5-[2-(4-Fluorophenyl) Pyridin-3-Yl]-1, 3, 4-Thiadiazol-2-Amine

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ABSTRACT

This research has focused on the incorporation of the thiadiazole moiety into versatile pyridine ring because of their biological properties. In order to explore the possibilities of some altered biological action author envisaged that by designing the Schiff base derivatives of 1, 3, 4-thiadiazole moiety may exhibit anticancer properties. These novel 1,3,4-thiadiazole Schiff base compounds have been synthesized by microwave-assisted synthesis and screened for their cytotoxicity on *HeLa*, *HepG2* and *MCF7* cancer cell lines.The key intermediate 2-(4-fluorophenyl)pyridine-3-carboxylic acid was obtained by hydrolysing the ester **3** in presence of KOH and methanol.Thus obtained compound **4** was treated with thiosemicarbazide and phosphorous oxychloride and cyclized in microwave inorder to get the intermediate 5-[2-(4-fluorophenyl) pyridin-3-yl]-1, 3, 4-thiadiazol-2-amine. The amine **5** was reacted with different aldehydes (**a-h**) in presence of catalytic amount of acetic acid and obtaineda series of novel Schiff base derivatives **6a-6h**. These compounds were characterized by MS, ^{1}H-NMR,IR and elemental analysis. Most of the compounds in this series have exhibited moderate cytotoxicity onall the three human cell lines at different concentrations, but two compounds **6f** and **6h** showed good inhibition towards liver carcinoma cell lines having IC_{50} of 23.8µMand 13.4µM respectively.

Keywords: carcinoma cell, thiadiazole

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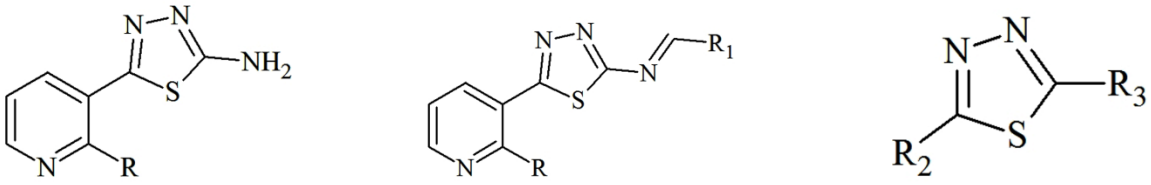
INTRODUCTION

The 1, 3, 4-thiadiazoles form a biologically important group of compounds having activities such as anti-inflammatory[1],anti-microbial [2, 3],anticancer [4], anti-hypertensive [5]. The modification in the structure of the pyridine ring by introducing 4-fluorophenylgroup at second position enhances the potency of the 1,3,4-thiadiazoles.Different Schiff base derivatives containing pyridine moiety(Figure 1, B)attract much interest due to its synthetic as well as various biological activities[6]. In view of the above properties of 1, 3, 4-thiadiazoles we have synthesized various novel Schiff basemolecules [7] that might possess anticancer properties. These compounds were obtained by the cyclisation of 2-(4-fluorophenyl) pyridine-3-carboxylic acid **4** in presence of thiosemicarbazide and phosphorous oxytrichloride under microwave irradiation and obtained amine(Figure 1, A).The amine **5** was further converted into stable Schiff base derivatives by reacting with various aldehydes(**a-h**) in presence of acetic acid and obtained the final products **6a-6h**.These types of novel ring systems have not been studied yet.The Schiff base compounds having 1, 3, 4-thiadiazole ring at third position of the pyridine systemcontaining 4-fluoro phenyl group at the second position showed good anti-proliferative activity [8].In view of the above facts author envisaged that by modifying the structure of the 1, 3, 4-thiadiazole and substituting with different aromatic groups having more bioavailability may possess good anticancer properties.Author substituted 4-fluoro phenyl group at second position and synthesized novel Schiff base derivatives of5-[2-(4-fluorophenyl) pyridin-3-yl]-1, 3, 4-thiadiazol-2-amine. These Schiff base derivatives **6a-6h** showed cytotoxicity on cancer cell lines.According to the SAR studies of these 1, 3, 4-thiadiazole ring by introducing more water soluble groups in the ring systems enhances the bio availability and increases considerably TPSA of the molecules.This kind of novel ring systems enhances the activity as a contribution to the development of new anticancer drugs we have synthesized novel Schiff bases**6a-6h** having inhibition against *Hela, Hep-G2* and *MCF7*cell lines.



A B C

Figure 1: A) 2-Substituted pyridine containing 1,3,4-thiadiazole amine; B) 2-Substituted pyridine containing 1,3,4-thiadiazole Schiff base derivatives; C) 2,5-Disubstituted 1,3,4-thiadiazole derivatives.

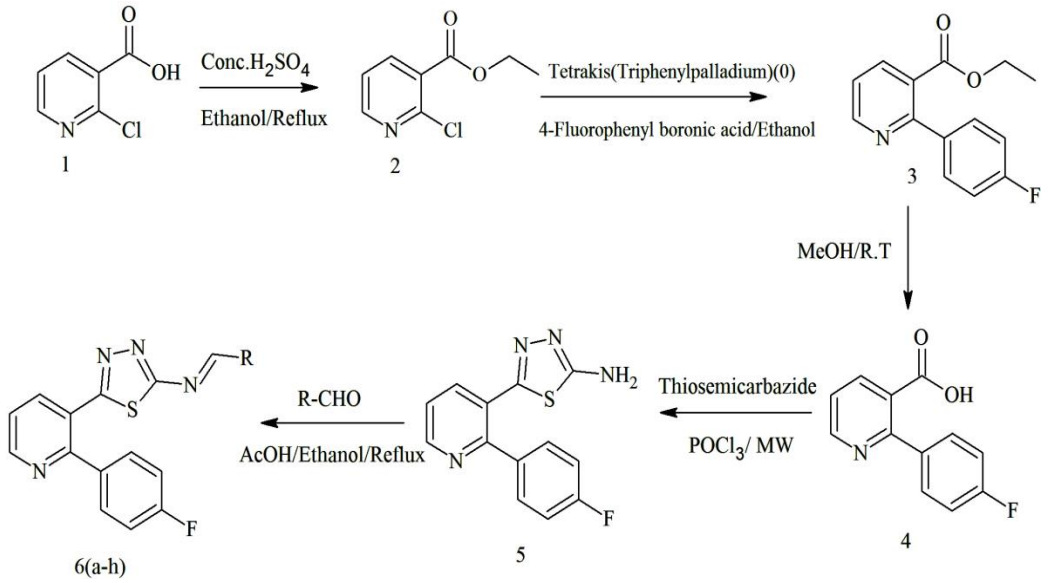


Figure 2: LinearSynthetic pathway of the Schiff base derivatives 5-[2-(4-Fluorophenyl) Pyridin-3-yl]-1, 3, 4-Thiadiazol -2-Amine.

EXPERIMENTAL

Synthesis of 2-Chloro-nicotinic acid ethyl ester: (2) (Figure 1)

The 2-chloronicotinicacid (10g, 0.0636mol) was taken in a 500mL single necked round bottom flask. Ethanol (150mL) was added under stirring.The round bottom flask was fitted with a water cooled condenser and kept on an oil bath. To the reaction mixture concentrated H_2SO_4 was added drop wise under stirring. The reaction mixture was refluxed at 80°C for 8 h.TLC (Thin layer chromatography) was monitored to check the progress of the reaction. Reaction was completed after 8h. RM was concentrated under reduced pressure to remove the solvent. Residue was cooled to 0°C and neutralized with saturated solution of $NaHCO_3$ and the product was extracted with ethyl acetate (2×25mL), washed with brine (10mL) and dried over Na_2SO_4.The solvent was removed completely under reduced pressure. The obtained ester was pure enough to carry forward to next step.

Synthesis of 2-(4-Fluoro-phenyl)-nicotinic acid ethyl ester (3)

Ethyl2-chloropyridine-3-carboxylate(8.5g, 0.0457mol), K_2CO_3(25.2g,0.183mol),4-fluorophenylboronic acid(7.0378g,0.0502mol) and tetrakis (triphenyl phosphine) palladium (0) (0.263g,304.8mol) were taken in a 250mL RB flask.Ethanol 100ml was added under nitrogen atmosphere. The RM was refluxed at 85°C for 10h.TLC was monitored to check the progress of the reaction. After completion, solvent was removed under reduced pressure. Ice cold water (100mL) was added and the aqueous was extracted with ethyl acetate (2×30mL), washed with brine (10mL) and dried over Na_2SO_4. Solvent was removed under reduced pressure. The crude

product (8.1g) was purified by column chromatography using silica gel (100 to 200mesh), gradient(0-15%) ethyl acetate in hexane as the eluent. Fractions were collected from the column and concentrated.

Synthesis of 2-(4-Fluoro-phenyl)-nicotinic acid (4)

Ethyl 2-(4-fluorophenyl) pyridine-3-carboxylate (6.8g, 0.0277mol) was taken in a 100mL single necked round bottom flask containing 50 mL of methanol. To this reaction mixture KOH (3.1g, 0.0555mol) was added and stirred at room temperature overnight.TLC was monitored to check the completion of the reaction.After completion, the methanol was removed under reduced pressure and residue was diluted with 50 mL of ice cold water. The residue was neutralized with concentrated HCl(P^{H}3to4) solid that are separated out was filtered, washed with cold water and dried.

Synthesis of 5-[2-(4-Fluoro-phenyl)-pyridin-3-yl]-[1, 3, 4] thiadiazol-2-yl amine (5)

2-(4-fluorophenyl) pyridine-3-carboxylicacid (4.2g, 0.0193mol) and thiosemicarbazide (2.641g, 0.0290mol) were taken in a 250mL microwave reaction flask. Phosphorous oxychloride(20mL) was added and irradiated with microwave (CEMsp-d discoverer model) for a period of 1.5 minutes(30sec/interval).The RM was neutralized with ammonia and bright yellow solid that are separated out was filtered, washed with water and dried.

General Procedure for the Synthesis of Schiff Base Derivatives of 5-[2-(4 Fluorophenyl) Pyridin-3-yl]-1, 3, 4-Thiadiazol-2-Amine.

200mg of the obtained amine (**5**) (1 equivalent), different substituted aromatic aldehydes (1.1.eqivalent) and 5 to 8 drops of acetic acid were added to the 100mL RB flask. Ethanol (10-20mL) was added and RM was refluxed at 80°C for 3 to 6h. TLCwas monitored to check the completion of the reaction. After the completion of the reaction, solvent was removed completely, residue was added with crushed ice and solids that were separated out was filtered, washed with water and dried. The crude product was purified by column chromatography using silica gel 100-200mesh and gradient used (0-70%) ethyl acetate in hexane.

MATERIALS AND METHOD

All the chemicals, Reagents and solvents were purchased from s-d fine and spectrochem chemicals.Mass spectra of the compounds were analysed using Agilent LCMS analyser (6420 series, Quadra pole). ^{1}H NMR and ^{13}CNMR of all the compounds were analysed using brucker 400MHz.All the IR spectroscopic analysis was done using FTIR tracer 100 series. The microwave reactions are carried out using whirlpool semi-automated microwave oven.

Table 1: *In-vitro* anticancer activity data of the synthesized 1, 3, 4-thiadiazole Schiff bases.

Compounds 6a-6h	IC_{50} values of the synthesized novel 1,3,4-thiadiazole Schiff bases (µM)		
	HeLa	***Hep-G2***	***MCF 7***
6a	96.5	350.4	123.5
6b	405.7	654.3	108.9
6c	232.4	213.6	459.6
6d	561	156.5	453.6
6e	567.8	123.6	89.9
6f	212.5	**23.8**	126.7
6g	102.5	453	678.7
6h	642	**13.4**	200.5
5-FU	8.2	7.4	7.8

IC_{50}-The inhibitory concentration of the compounds at 50%.

Preparation of the Cell culture and Fixation.:

All the three cell lines were grown in RPMI 1640 medium containing 10% of fetalbovinserum.This was added with antibiotics (100 units/mL penicillin, 100µg/mL streptomycin, L-glutamine 0.03%, w/v and sodium bicarbonate 2.2%, w/v). All the cell lines were cultured in flasks kept in a 100% humidified atmosphere incubated with 10% of CO_2 at 37^0C for 24h. Before the addition of the synthesized 1,3,4-thiadiazoles after 24h, the aliquots of 10 µL the test compounds (100µg/mL, 250µg/mL, 500µg/mL and maximum of 1000µg/mL) of different dilutions were added to the appropriate micro-titre plate containing the 90µl of cells. Subcultures were performed with 0.05% trypsin and 0.02% EDTA in phosphate-buffered saline solution.

MTT assay:

The MTT assay was carried out in Genelon Institute of Life Sciences Pvt.Ltd according to the method set out by Mossman. The cells lines were plated into 96-well microtiter plates at a density of 1×10^4 cells/well. After 24 h, the culture medium was replaced with 200 µL RPMI 1640 medium supplemented with 10% fetal bovine serum containing varying concentrations. The cells were then subjected to incubation for 24h and 48 h. The final concentration of solvent was less than 0.1% in the cell culture medium. The culture solutions were removed and replaced with 90 µL of culture medium. Ten microliters of sterile filtered MTT (Sigma, USA) solution (5 mg/mL) suspended in PBS (pH = 7.4) was added to each well to achieve a final concentration of 0.5 mg MTT/mL. The cells were then incubated at 37°C for 4 h. After the medium and unreacted dye was removed, 200 µL of DMSO was added to each well. The absorbance at 490 nm of the dissolved solution was measured using a Bio-Rad 680 micro plate reader (BIORAD, USA).The relative cell viability (%) of the control wells containing cell culture medium without the tested compound was calculated by

dividing the absorbance of treated cells by that of the controls in each experiment. The IC_{50} was calculated by using statistical software, which inhibits the growth of 50% of cells in relation to non-treated control cells.

RESULTS AND DISCUSSION

Analytical Data of the Intermediate compound (2)

Yield 8.5g; ms (ESI) *m/z*: [M+H]- 187; HPLC96%; TLC-ethyl acetate: hexane (1:9); IR (KBr), ν_{max}/cm^{-1} : 980 (C-O stretching esters, w),1089(C-Cl stretching, w), 2845 (C-H stretching, s), 3006(C-H stretching, w); ^{1}HNMR ($CDCl_3$, 400MHz) -δ 1.15(t, 3H), 3.85(q, 2H), 7.4(t, 1H), 8.45 (dd,*J* 13.5Hz 1H), 8.85(d, *J* 8.5Hz, 1H).

Analytical Data of the Intermediate compound (3)

Yield 6.8g; colourless syrup;ms (ESI) *m/z*: [M+H]-246; IR (KBr), ν_{max}/cm^{-1} : 880(C-F stretching, w), 1100(C-O stretching esters, w),2945(C-H stretching, s), 3106(C-H stretching, w); HPLC 94%; ^{1}H-NMR($CDCl_3$, 400MHz): δ0.8(t,2H), 3.5(q,3H), 7.15(dd,*J* 7.4Hz, 2H), 7.6(q,2H), 8.6(m,*J* 13.2Hz, 1H), 9.1(q,2H).

Analytical Data of the Intermediate compound (4)

Yield 4.2g; ms(ESI) *m/z*: [M+H]-216; pale brown solid; m.p-145-148°C; IR (KBr), ν_{max}/cm^{-1}) :920(C-F stretching, w), 1230 (OH bending, w), 2450(OH stretching),2915(C-H stretching, s), 3016(C-H stretching, w); HPLC 92.8%; ^{1}H-NMR($CDCl_3$, 400MHz):-δ 7.2(dd,*J* 8.5Hz, 2H), 7.6(dd,2H), 7.8(t,2H), 9.3(m,*J* 12.8Hz, 1H), 11.1(bs, 1H).

Analytical Data of the Intermediate compound (5)

Yield 2.5g; m.p-156-157°C; ms (ESI) *m/z*: [M-H]-270; IR (KBr),ν_{max}/cm^{-1}) : 910(C-F stretching, w),1280 (CN stretching, m),1480(NH bending), 2905(C-H stretching, s), 3026(C-H stretching, w),3450(NH-stretching, s); HPLC 95.6%;^{1}H NMR($CDCl_3$, 400MHz) : δ 5.2(bs, 2H, NH_2), 7.25(dd,*J* 8.6Hz, 2H), 7.5(t,1H), 7.75(dd,*J* 7.4Hz, 2H), 8.1(d, 2H).

The Analytical Data (M.P, IR,^{1}H-NMR) of The Novel Schiff Base Derivatives having 1, 3, 4-Thiadiazole Moiety:

(4-Chloro-benzylidene)-{5-[2-(4-fluoro-phenyl)-pyridin-3-yl]-[1,3,4]thiadiazol-2-yl}-amine

{6a}:R = 4-Chloro-benzylidene.

Pale yellow solid; yield, 67%; m.p-123-124°C; IR (KBr), ν_{max}/cm^{-1}: 910(C-F stretching, w),1008 (C-Cl stretching, s), 1290(CN stretching, m),2925(C-H stretching, s), 3046(C-H stretching, w), ^{1}H-NMR($CDCl_3$, 400MHz) -δ 7.35(dd,2H Ar-H), 7.6(dd, *J* 12.5Hz, 2H), 7.8(dd,2H), 8.25(d,*J* 13.2Hz, 2H), 9.15(m,2H); ^{13}CNMR ($CDCl_3$, 100MHz): 116, 124, 126.5, 129, 129.5, 130, 135,

137.5, 156.5, 155, 162.3, 163; ms (ESI) *m/z*: [M+H] 383; HPLC 94.5%;molecular formula- $C_{19}H_{11}ClFN_4S$;anal.calculated: C, 59.77; H, 2.90; Cl, 9.28; F, 4.98; N, 14.67; S, 8.40; found C, 59.75; H, 2.94; Cl, 9.31; F, 4.95; N, 14.65; S, 8.45.

{5-[2-(4-Fluoro-phenyl)-pyridin-3-yl]-[1,3,4]-thiadiazol-2-yl}-(4-methoxy-benzylidene)-amine **{6b}**: **R = 4-methoxy-benzylidene.**

Pale yellow solid; yield 56%; m.p-142-143°C; IR (KBr), ν_{max}/cm^{-1}: 910(C-F stretching, w),1120 (CS stretching, s), 1310 (CN stretching, m), 2905(C-H stretching, s), 3034(C-H stretching, w); ^{1}H-NMR($CDCl_3$, 400MHz): δ 2.3(s, 3H, OCH_3), 7.23(dd,*J* 8.5Hz, 2H,Ar-H), 7.35(dd,2H), 7.6(m,*J* 7.8Hz, 3H), 7.8(dd, 2H), 8.23(d,*J* 8.4Hz, 2H); ^{13}CNMR ($CDCl_3$, 100MHz): 65, 114, 116, 120.8, 124.3, 129, 129.5, 135, 137, 150.5, 155, 160, 162.5, 163;ms(ESI) *m/z*: [M+H] 391; HPLC 96.4%; molecular formula- $C_{21}H_{15}FN_4OS$; anal. calculated: C, 64.60; H, 3.87; F, 4.87; N, 14.35; O, 4.10; S, 8.21; found C, 64.50; H, 3.89; F, 4.86; N, 14.32; O, 4.20; S, 8.31.

(4-Dimethylamino-benzylidene)-{5-[2-(4-fluoro-phenyl)-pyridin-3-yl]-[1,3,4]thiadiazol-2-yl}-amine {**6c**}:**R =4-Dimethylamino-benzylidene.**

Brown coloured solid; yield 76%; m.p-143-144°C; IR (KBr), ν_{max}/cm^{-1}: 940(C-F stretching, w),1020 (NC-Stretching, s), 1300 (CN stretching, m), 2825(C-H stretching, s), 2946(C-H stretching, w), ^{1}H-NMR($CDCl_3$, 400MHz): δ 1.8(s,6H, NCH_3), 7.1(dd,*J* 12.5Hz, 2H,Ar-H), 7.32(dd,2H), 7.7(m,3H), 7.8(dd,2H), 8.3(d,*J* 8.5Hz, 2H); ^{13}C NMR ($CDCl_3$, 100MHz): 45.5, 113, 116, 124.5, 129.5, 135, 137, 150, 150.5, 160, 162.5, 163;ms(ESI) *m/z*: [M+H] 404; HPLC 95.1%; molecular formula- $C_{22}H_{18}FN_5S$; anal. calculated: C, 65.49; H, 4.50; F, 4.71; N, 17.36; S, 7.95;found C, 64.49; H, 4.54; F, 4.75; N, 17.32; S, 7.97.

(5-Bromo-2-fluoro-benzylidene)-{5-[2-(4-fluoro-phenyl)-pyridin-3-yl]-[1,3,4]thiadiazol-2-yl}-amine:{ **6d**}:**R = 5-Bromo-2-fluoro-benzylidene**.

Off white coloured solid; yield 65%; m.p-150-152°C; IR (KBr), ν_{max}/cm^{-1}: 680 (C-Br stretching, s), 950 (C-F stretching, w),1260 (C-F stretching, s), 1298 (CN stretching, m), 2845(C-H stretching, s), 3026 (C-H stretching, w); ^{1}H-NMR($CDCl_3$, 400MHz)-δ 7.25(dd,2H,Ar-H), 7.4(dd,*J* 12.5 Hz, 2H), 7.8(m,*J* 13.6Hz, 3H), 8.2(d,*J* 8.2Hz, 2H), 9.05(d, 2H); ^{13}CNMR ($CDCl_3$, 100MHz): 116, 117, 118.5, 124, 129, 133.4, 135, 137, 155, 161.5, 162.3, 163; ms(ESI) *m/z*: [M+H]-459; HPLC 95.7%; molecular formula- $C_{20}H_{11}BrF_2N_4S$; anal. calculated: C, 52.53; H, 2.42; Br, 17.47; F, 8.31; N,12.25; S,7.01; found C, 52.43; H, 2.48; Br, 17.46; F, 8.34; N,12.26; S,7.11.

{5-[2-(4-Fluoro-phenyl)-pyridin-3-yl]-[1, 3, 4] thiadiazol-2-yl}-[5-(4-fluoro-phenyl)-thiophen-2-ylmethylene]-amine{**6e**}:**R= 5-(4-fluoro-phenyl)-thiophen-2-ylmethylene.**

Dark brown solid; yield 54%; m.p-102°C; IR (KBr), ν_{max}/cm^{-1}: 980(C-F stretching, w),1120 (CS stretching, m), 1208(CN stretching, m),1264 (C-F stretching, s), 2895(C-H stretching, s), 3015(C-H stretching, w); ^{1}H-NMR-($CDCl_3$, 400MHz):*δ* 7.45(dd,*J* 8.7Hz,Ar-H), 7.5(dd,2H), 7.7(m,3H), 7.9(dd,*J* 8.5Hz, 2H), 8.3(d,2H), 9.1(d,*J* 7.6Hz, 2H); ^{13}CNMR ($CDCl_3$, 100MHz): 116, 124.5, 126, 129, 135, 136, 137.5, 150, 155, 163; ms: (ESI)*m/z*:[M+H]-462; HPLC 96.8%; molecular formula- $C_{24}H_{14}F_2N_4S_2$:anal. calculated: C, 62.59; H, 3.06; F, 8.25; N, 12.17; S, 13.93; found C, 62.56; H, 3.09; F, 8.15; N, 12.27; S, 13.95.

(5-Bromo-thiophen-2-ylmethylene)-{5-[2-(4-fluoro-phenyl)-pyridin-3-yl]-[1,3,4]thiadiazol-2-yl}-amine {**6f**}; R = **5-Bromo-thiophen-2-ylmethylene.**

Brown solid; yield 55%; m.p-118-119°C; IR (KBr), ν_{max}/cm^{-1}: 718(C-Br stretching, s),1050(C-F stretching, w), 1210 (C-F stretching, s), 1218 (CN stretching, m), 2825(C-H stretching, s), 2926(C-H stretching, w); ^{1}H-NMR($CDCl_3$, 400MHz) : *δ* 7.21(dd,2H), 7.35(dd,*J* 13.2Hz, 2H), 7.7(m,2H), 7.8(dd,2H), 8.25(d,*J* 8.6Hz, 2H);^{13}CNMR ($CDCl_3$, 100MHz): 116, 193, 120, 126, 129, 135, 137, 138, 145, 150, 155, 163;ms(ESI)*m/z*:[M+H] 447; HPLC 97.3%; molecular formula- $C_{18}H_{10}BrFN_4S_2$; anal. calculated: C, 48.55; H, 2.26; Br, 17.94; F, 4.27; N, 12.58; S, 14.40; found C, 48.45; H, 2.23; Br, 17.84; F, 4.17; N, 12.53; S, 14.35.

{5-[2-(4-Fluoro-phenyl)-pyridin-3-yl]-[1,3,4]thiadiazol-2-yl}-(3-methyl-thiophen-2-ylmethylene)-amine {**6g**}: **R = 3-methyl-thiophen-2-ylmethylene.**

Off white coloured solid; yield66%; m.p-130-131°C; IR(KBr), ν_{max}/cm^{-1}: 1050(C-F stretching, w),1198(CN stretching, m),1310(C-F stretching, s), 2825 (C-H stretching, s), 3010 (C-H stretching, w);^{1}H-NMR($CDCl_3$, 400MHz) :*δ* 1.3(s, 3H, Thiophene CH_3), 7.35(dd,2H), 7.5(q,2H, Ar-H), 8.2(d,*J* 13.5Hz, 2H), 9.1(dd,*J* 8.5Hz, 2H); ^{13}CNMR ($CDCl_3$, 100MHz): 20, 116, 124, 125, 126, 127.8, 129, 135, 137, 144.3, 145, 150, 155, 163; ms(ESI)*m/z*: [M+H]-381; HPLC 98.2%; molecular formula- $C_{19}H_{13}FN_4S_2$;anal.calculated: C, 59.98; H, 3.44; F, 4.99; N, 14.73; S, 16.86; found C, 59.88; H, 3.43; F, 4.94; N, 14.83; S, 16.84.

(4'-Fluoro-biphenyl-2-ylmethylene)- {5-[2-(4-fluoro-phenyl)-pyridin-3-yl]-[1,3,4]thiadiazol-2-yl}-amine {**6h**}: **R =4'-Fluoro-biphenyl-2-ylmethylene.**

Pale brown solid; yield 78%; m.p-121-125°C; IR (KBr), ν_{max}/cm^{-1}: 920(C-F stretching, w),1303 (C-F stretching, s), 1318(CN stretching, m),2818(C-H stretching, s), 2896(C-H stretching, w); ^{1}H-NMR($CDCl_3$, 400MHz) : *δ* 7.43(dd,2H Ar-H), 7.7(m,*J* 13.5Hz, 2H), 7.9 (m, 3H), 8.3 (d, *J* 8.5Hz, 4H), 9.15 (dd, *J* 12.4Hz, 4H);^{13}CNMR ($CDCl_3$, 100MHz): 116, 124, 129, 135, 137.6, 141.5, 150, 160.3, 162, 163; ms (ESI) *m/z*: [M+H] 428; HPLC 98.2%; molecular formula- $C_{26}H_{16}F_2N_4S$; anal.

calculated; C, 68.71; H, 3.55; F, 8.36; N, 12.33; S, 7.06; found C, 68.61; H, 3.65; F, 8.36; N, 12.23; S, 7.04.

Chemistry:

The 2-chloronicotinic acid **1**was converted into ethyl-2-chlorobenzoate by using ethanol and concentrated H_2SO_4[9].The ester**2** was reacted with 4-fluorophenyl boronic acid in presence of tetrakis(triphenyl phosphine) palladium (0)catalyst(Suzuki coupling)obtained the compound**3** (**Figure 2**).The benzoate was converted into corresponding acid**4** by treating alkaline methanol at room temperature(**Figure 2**). The acid thus obtained was cyclised in presence of phosphorous oxy chloride and thiosemicarbazideyielded the intermediate 5-[2-(4-fluorophenyl) pyridin-3-yl]-1, 3, 4-thiadiazol-2-amine [10]. (**Figure 2**)The amine**5**was reactedwith different aldehydes (**a-h**) in presence of acetic acid and ethanol and obtained the final Schiff base compounds **6a-6h**.

SAR(Structural Activity Relationship):

Structural activity relationship studies showed that the compounds containing 1, 3, 4-thiadiazole exhibits wide range of pharmacological properties. The design of the compounds made such that the compounds may showanticancer properties. According to the literature of pyridine moiety having various substitutions at different position shows wide range of pharmacological properties such as anthelmintic[11], anticancer[12, 13]and anticonvulsant[14] activities. In this work author has synthesized novel derivatives in which second position of the pyridine ring contains 4-fluorophenyl group which might overcome the water insolubility problem thus increasing bioavailability and may enhance the anticancer properties. Keeping in view of the 4-fluorophenyl ring at second position author has constructed1, 3, 4-thiadiazole amine [15, 16]at third position which might possesses good anticancer properties. Further author envisaged that by making Schiff bases compounds with different substituted aromatic groups may further enhance the TPSA (Total polar surface area) of the molecule and whereby overcoming the water insolubility problem of Schiff base compounds thus showing good anti-proliferative activity.

Anticancer Properties (Table 1):

The synthesized novel 1, 3, 4-thiadiazole Schiff base compounds were screened for their *invitro*cytotoxicity i.e. MTT assay [17]on three leukemic cell lines. The *in vitro* anticancer activity has been done on three human cell lines namely, (a) Human cervical carcinoma cell line(***HeLa***), (b) Human liver carcinoma cell lines(***HepG2*)**, (c) Human breast carcinoma cell lines(***MCF7***).The results were expressed in the form of IC_{50} (Inhibitory concentration of the compound at 50%). The compounds (**6a**), (**6b**) and (**6g**) were showed moderate inhibition on ***HepG2*** cell line. The compounds (**6b**), (**6c**), (**6d), (6e**) and (**6h**) were less cytotoxic on ***Hela*** carcinoma cell lines.

Whereas the compounds (**6a**), (**6f**) and (**6h**) are moderately cytotoxic to ***Hela*** cell lines. Compounds (**6c**), (**6d**) and (**6g**) were less cytotoxic on***MCF7*** cell lines. Interestingly the two compounds containing 1, 3, 4-thiadiazole ring having substitution (**R**)of 5-bromothiophene and biphenyl compound (**6f**) and (**6h**) showed good inhibition on liver carcinoma cell line(*HepG2*) having IC_{50} of 23.8µM and 13.4µM respectively.

CONCLUSION

In this synthetic scheme the author has synthesized various novel derivatives of Schiff base compounds containing 1, 3, 4-thiadiazole ring and purified these compounds by column chromatography. The purity of these compounds were in the range of 95-97%. These compounds were subjected to anti-proliferative assay (MTT) on human carcinoma cell lines at different concentrations and obtained IC_{50} values.The IC_{50} values of the 1, 3, 4-thiadiazole Schiff base derivatives have been compared with the IC_{50} of the standard 5-FU. In this research the author has found out that concentration of the compounds required to inhibit the cell lines used in the experiment were high and compounds (**6f**) and (**6h**) showed good *invitro* cytotoxicity as compared with the 5-FU.Compounds **6f** and **6h** were showed good cytotoxicity on liver carcinoma cell line (*HepG2*) exhibiting IC_{50} of 23.8µM and 13.4µM respectively. Further experiments with different cell lines were in progress.

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