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Research Paper

Antiproliferative Activity Of New Schiff Bases Derived From 3-Fluoro-5-(Trifluoromethyl)Benzylamine

Chemistry

KEYWORDS : 3-Fluoro-5-(trifluoromethyl)benzylamine, Aldehydes, MTT assay, Antiproliferative activity

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ABSTRACT

A series of new 3-fluoro-5-(trifluoromethyl)benzylamine derivatives 3(a-i) were synthesized and characterized by different spectral studies. New compounds were evaluated for their antiproliferative effect using the MTT assay method against two human cancer cell lines (MCF7 and U373) and one astrocytoma brain tumor (C6 rat glioma) cell line. Among the series, compounds 3e and 3i showed good activity on all cell lines, whereas the other compounds in the series exhibited moderate activity.

1. Introduction

Schiff bases derived from amines and aldehydes have a wide variety of applications in many fields, e.g., biological, inorganic and analytical chemistry [1-5]. They are used in optical and electrochemical sensors, as well as in various chromatographic methods, to enable detection of enhance selectivity and sensitivity [6-8]. In recent years there has been an increased interest in the application of medical treatment as information is constantly gathered linking the development of human diseases. Biological activity of Schiff bases have been reported and they are active against a wide range of organisms [9, 10].

The unique character of fluorinated molecules is well known in medicinal chemistry and agro chemistry [11-15]. The introduction of fluorine into organic molecules may increase the lipophilicity and thus enhance the rate of cell penetration and transport of a drug to an active site. Fluorine as a unique atom can modulate the physical properties of a molecule and the fungicidal activity was often enhanced by introducing fluorine into molecules [16, 17]. Flusilazole, fluotrimazole, epoxiconazole and flutriafol, which are successful examples of fluorinated 1,2,4-triazole fungicides have been widely used in plant protection [18, 19]. As a result, we focused to synthesize a series of fluorine containing Schiff bases 3(a-i) derived from 3-fluoro-5-(trifluoromethyl)benzylamine (1) and aldehydes 2(a-i) and evaluate them for Antiproliferative activity.

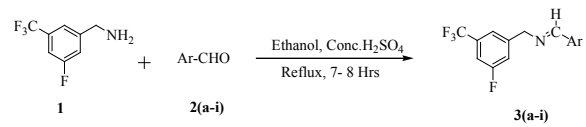
2. Experimental

2.1. Materials and reagents

All solvents and reagents were purchased from Merck Chemicals. The elemental analyses of the compounds were performed on a Perkin Elmer 2400 elemental analyser. The UV-Visible spectrum was recorded on UV-1800 SHIMADZU UV spectrometer with quartz cell of 1.0 cm path length. The FT-IR spectra was recorded using KBr discs on Jasco FT-IR 4100 infrared spectrophotometer. The ^{1}H NMR spectra was recorded using *Bruker DRX* 400 spectrometer at 300 *MHz* with tetramethylsilane as the internal standard. *Mass spectral data was obtained* by *LC/MSD Trap XCT*.

2.2. General procedure for the synthesis of 3-fluoro-5-(trifluoromethyl)benzylamine derivatives 3(a-i)

Equimolar concentrations of 3-fluoro-5-(trifluoromethyl) benzylamine (**1**) and aryl aldehydes (**2a-i**) were stirred for 7-8 hr at room temperature in absolute ethanol (25 ml) and then 2-3 drops of conc. sulfuric acid was added to the mixture. The reaction completion was confirmed by TLC. The solvent was concentrated and the precipitated solid was filtered, dried and recrystallized from methanol. 3-Fluoro-5-(trifluoromethyl)benzylamine derivatives **3(a-i)** were synthesized by the method summarized in Scheme 1.



1 **2(a-i)** **3(a-i)**

Scheme 1

2.2.1. Synthesis of (E)- N-benzylidene (3- fluoro-5-(trifluoromethyl) phenyl) methanamine (3a)

FT-IR (KBr, cm^{-1}): 3079 (Ar-H), 1669 (HC=N), 1463 (C=C), 1103 (C-F). ^{1}H NMR (DMSO-d$_6$) δ ppm: 4.82 (s, 2H, CH$_2$), 7.07-7.21 (m, 3H, Ar-H), 7.29 (m, 3H, Ar-H), 7.62 (d, 2H, Ar-H), 8.18 (s, 1H, CH=N). MS (ESI) *m/z*: 281.25. Anal. Calcd. for $C_{15}H_{11}F_4N$ (in %): C, 64.06; H, 3.94; N, 4.98. Found: C, 37.29; H, 2.14; N, 10.85.

2.2.2. Synthesis of (E)-N-(4-chlorobenzylidene)(3-fluoro-5-(trifluoromethyl)phenyl) methanamine (3b)

FT-IR (KBr, cm^{-1}): 3083 (Ar-H), 1675 (HC=N), 1468 (C=C), 1106 (C-F), 718 (C-Cl). ^{1}H NMR (DMSO-d$_6$) δ ppm: 4.88 (s, 2H, CH$_2$), 7.01-7.15 (m, 3H, Ar-H), 7.30 (d, 2H, Ar-H), 7.56 (d, 2H, Ar-H), 8.23 (s, 1H, CH=N). MS (ESI) *m/z*: 315.69. Anal. Calcd. for $C_{15}H_{10}ClF_4N$ (in %): C, 57.07; H, 3.19; N, 4.44. Found: C, 57.11; H, 3.25; N, 4.40.

2.2.3. Synthesis of (E)-N-(2-bromobenzylidene)(3-fluoro-5-(trifluoromethyl)phenyl) methanamine (3c)

FT-IR (KBr, cm^{-1}): 3078 (Ar-H), 1671 (HC=N), 1464 (C=C), 1110 (C-F), 527 (C-Br). ^{1}H NMR (DMSO-d$_6$) δ ppm: 4.79 (s, 2H, CH$_2$), 7.02-7.11 (m, 3H, Ar-H), 7.19 (t, 1H, Ar-H), 7.29 (t, 1H, Ar-H), 7.41 (d, 1H, Ar-H), 7.50 (d, 1H, Ar-H), 8.48 (s, 1H, CH=N). MS (ESI)

m/z: 360.14. Anal. Calcd. for $C_{15}H_{10}BrF_4N$ (in %): C, 50.02; H, 2.80; N, 3.89. Found: C, 50.05; H, 2.87; N, 3.92.

2.2.4. Synthesis of E)-N-(4-nitrobenzylidene)(3-fluoro-5-(trifluoromethyl)phenyl) methanamine (3d)

FT-IR (KBr, cm^{-1}): 3061 (Ar-H), 1668 (HC=N), 1459 (C=C), 1159 (C-N), 1119 (C-F). ^1H NMR (DMSO-d_6) δ ppm: 4.95 (s, 2H, CH_2), 7.57-7.62 (m, 3H, Ar-H), 8.06-8.09 (m, 2H, Ar-H), 8.31-8.34 (m, 2H, Ar-H), 8.71 (s, 1H, CH=N). MS (ESI) *m/z*: 326.25. Anal. Calcd. for $C_{15}H_{10}F_4N_2O_2$ (in %): C, 55.22; H, 3.09; N, 8.59. Found: C, 55.25; H, 3.05; N, 8.62.

2.2.5. Synthesis of (E)-N-(3,5-dinitrobenzylidene)(3-fluoro-5-(trifluoromethyl)phenyl) methanamine (3e)

FT-IR (KBr, cm^{-1}): 3064 (Ar-H), 1677 (HC=N), 1461 (C=C), 1151 (C-N), 1101 (C-F). ^1H NMR (DMSO-d_6) δ ppm: 4.98 (s, 2H, CH_2), 7.11-7.25 (m, 3H, Ar-H), 8.84 (s, 2H, Ar-H), 9.07 (s, 1H, Ar-H), 8.86 (s, 1H, CH=N). MS (ESI) *m/z*: 371.24. Anal. Calcd. for $C_{15}H_9F_4N_3O_4$ (in %): C, 48.53; H, 2.44; N, 11.32. Found: C, 48.49; H, 2.47; N, 11.35.

2.2.6. Synthesis of (E) -N -(4 -(methylthio) benzylidene) (3-fluoro-5-(trifluoromethyl)phenyl) methanamine (3f)

FT-IR (KBr, cm^{-1}): 3076 (Ar-H), 1680 (HC=N), 1468 (C=C), 1298 (C-S), 1098 (C-F). ^1H NMR (DMSO-d_6) δ ppm: 2.69 (s, 3H, CH_3), 4.82 (s, 2H, CH_2), 7.26-7.28 (m, 3H, Ar-H), 7.69-7.71 (m, 2H, Ar-H), 7.75-7.78 (m, 2H, Ar-H), 8.36 (s, 1H, CH=N). MS (ESI) *m/z*: 327.34. Anal. Calcd. for $C_{16}H_{13}F_4NS$ (in %): C, 58.71; H, 4.00; N, 4.28. Found: C, 58.65; H, 4.03; N, 4.31.

2.2.7. Synthesis of 4-((E)-(3-fluoro-5-(trifluoromethyl)benzylimino)methyl)-N,N-dimethyl benzenamine (3g)

FT-IR (KBr, cm^{-1}): 3081 (Ar-H), 1671 (HC=N), 1469 (C=C), 1151 (C-N), 1108 (C-F). ^1H NMR (DMSO-d_6) δ ppm: 2.89 (s, 6H, CH_3), 4.79 (s, 2H, CH_2), 6.69 (d, 2H, Ar-H), 7.05-7.18 (m, 3H, Ar-H), 7.52 (d, 2H, Ar-H), 8.71 (s, 1H, CH=N). MS (ESI) *m/z*: 324.32. Anal. Calcd. for $C_{17}H_{16}F_4N_2$ (in %): C, 62.96; H, 4.97; N, 8.64. Found: C, 62.93; H, 4.93; N, 8.67.

2.2.8. Synthesis of 4-((E)-(3-fluoro-5-(trifluoromethyl)benzylimino)methyl)-2-methoxyphenol (3h)

FT-IR (KBr, cm^{-1}): 3514 (O-H), 3059 (Ar-H), 1660 (HC=N), 1454 (C=C), 1106 (C-F). ^1H NMR (DMSO-d_6) δ ppm: 3.69 (s, 3H, CH_3), 4.85 (s, 2H, CH_2), 7.61 (d, 1H, Ar-H), 6.85 (s, 1H, Ar-H), 6.91 (d, 1H, Ar-H), 7.01-7.09 (m, 3H, Ar-H), 8.39 (s, 1H, CH=N), 9.95 (d, 1H, Ar-OH). MS (ESI) *m/z*: 327.27. Anal. Calcd. for $C_{16}H_{13}F_4NO_2$ (in %): C, 58.72; H, 4.00;N, 4.28. Found: C, 58.78; H, 4.05;N, 4.31.

2.2.9. Synthesis of (E)-N-((1H-indol-3-yl)methylene)(3-fluoro-5-(trifluoromethyl)phenyl) methanamine (3i)

FT-IR (KBr, cm^{-1}): 3173 (N-H), 3075 (Ar-H), 1679 (HC=N), 1468 (C=C), 1117 (C-F). ^1H NMR (DMSO-d_6) δ ppm: 4.62 (s, 2H, CH_2), 7.05-7.18 (m, 3H, Ar-H), 7.31 (s, 1H, Ar-H), 7.45-7.68 (m, 4H, Ar-H), 8.72 (s, 1H, CH=N) 10.05 (s, 1H, indole-NH).MS (ESI) *m/z*: 320.28. Anal. Calcd. for $C_{17}H_{12}F_4N_2$ (in %): C, 63.75; H, 3.78; N, 8.75. Found: C, 63.78; H, 3.81; N, 8.74.

2.3. In vitro **antiproliferative activity**

2.3.1. Drugs and solutions

The 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was dissolved (5 mg/ml) in phosphate buffer saline pH 7.2 and filtered (0.22 ml) before use. The RPMI 1640 cell culture medium, MTT and fetal bovine serum (FBS) were purchased from Merck chemicals.

2.3.2. Cell lines and culture conditions

MCF7, U373 and C6 rat glioma cell lines were procured from National Center for Cell Sciences, Pune, India. All cells were grown in RPMI-1640 supplemented with 10 % heat inactivated FBS, 100 IU/ml penicillin, 100 mg/ml streptomycin and 2 mM-glutamine. Cultures were maintained in a humidified atmosphere with 5 % CO_2 at 37 °C. The cells were subcultured twice each week, seeding at a density of about 2×10^3 cells/ml.

2.3.3. In vitro cell viability assay-MTT assay

The potential effects on cell viability were investigated by using the MTT assay [20]. It is an indicator of metabolically active cells. A known number of MCF7, U373 and C6 rat glioma cells were transferred into 96 well plates in a volume of 200 µl of culture medium and incubated for 48 h before addition of test compound. Cells were then exposed to known concentrations of the compound to be tested (100 µM, 200 µM and 400 µM) for 24 h at 37 °C. After drug exposure, the culture medium was removed and 20 µl of MTT reagent (diluted in culture medium, 5 mg/ml) was added. After incubating for 4 h, the MTT/medium was removed and DMSO (100 µl) was added to each well and plates were agitated for 1 min. Absorbance of the coloured solution was measured on a multi-well plate reader (Victor3, Perkin Emler) using a test wavelength of 570 nm. Results were evaluated by comparing the absorbance of the wells containing compound treated cells with the absorbance of wells containing 0.1 % DMSO alone (solvent control). Conventionally, cell viability was estimated to be 100 % in the solvent control and assay was performed in triplicate.

3. Results and discussion

3.1. Chemistry

The chemical structures and physical data of all the synthesized compounds are tabulated in Table 1. The 3-fluoro-5-(trifluoromethyl)benzylamine (**1**) was reacted with various aldehydes (R-CHO, **2a-i**) in ethanol to obtain 3-fluoro-5-(trifluoromethyl)benzylamine derivatives **3(a-i)** in good yield (65-81 %). The UV spectra of **3(a-i)** were recorded using suitable solvents in the range of 200 - 800 nm. The electronic absorption spectra of compounds show new bands and appearance of wavelength absorption band in the UV region in UV-visible spectrum owing to confirms the formation of new compounds. The FT-IR spectra of **3(a-i)** were recorded using KBr pellets in the range of 4000 - 400 cm^{-1}. The absorption bands at 3059 - 3083 cm^{-1} are assigned to the aromatic-H stretch. The appearance of a medium to strong absorption bands at 1660-1680 cm^{-1} due to a stretching vibration of the azomethine (HC=N) bond formation in the synthesized compound. New bands appeared at 1098 - 1119 cm^{-1} corresponding to C-F stretching frequency. The strong bands at 718 cm^{-1} and 527 cm^{-1} are assigned to the C-Cl and C-Br stretch in **3b** and **3c**, respectively. The proton spectral data of the starting material, 3-fluoro-5-(trifluoromethyl)benzylamine **1** shows resonance at δ 5.52 ppm (s, 2H, $-NH_2$). In all the synthesized compounds **3(a-i)**, the above resonance disappeared and additional resonances assigned to the –CH=N (δ 8.18 – 8.86 ppm) were observed which confirmed the product. The mass spectra of **3a** showed molecular ion peak at m/z 281.25 which is in agreement with the molecular formula $C_{15}H_{11}F_4N$. The elemental analyses data showed good agreement between the experimentally determined values and the theoretically calculated values within ± 0.4 %.

3.2. Antiproliferative activity

The antiproliferative action of the synthesized compounds **3(a–i)** were tested against three different cell lines. The activity was evaluated by measuring the levels of surviving cells after incubation for 24 h with the test samples using the MTT colorimetric assay based on the ability of metabolically active cells to convert the pale yellow MTT to a blue formazan product which is quantifiable spectrophotometrically. The percentage cell survival for tested compounds against human cancer cells (MCF7 and U373) and astrocytoma brain tumor (C6 rat glioma) cells are tabulated in Table 2. The results were expressed as percentage of cell proliferation compared with cells in control (cells treated with vehicle, 0.1% DMSO). Compound **3i** containing indole group and **3e**

containing nitro groups are more potent antiproliferative activity. Antiproliferative activity of **3a, 3b, 3c, 3d, 3f, 3g** and **3h** in the series showed moderate activity.

4. Conclusion

In conclusion, a series of new Schiff bases **3(a-i)** were synthesized and their antiproliferative activity has been evaluated Compounds **3e** and **3i** are shown to be more antiproliferative activity. From this work, we were able to identify a few active molecules which are capable of inhibiting the growth of cancer cell lines *in vitro*.

Acknowledgments

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Table 1 Chemical structure and Physical data of the synthesized compounds 3(a-i)

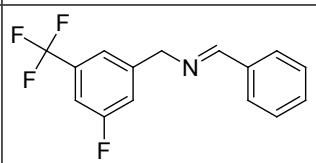
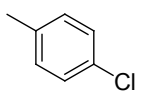
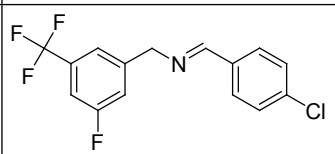
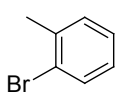
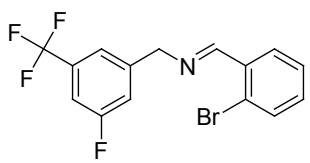
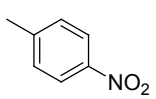
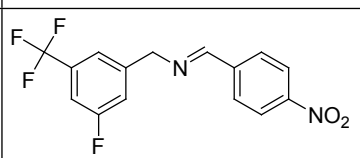
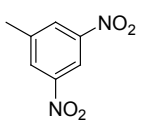
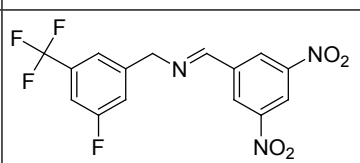
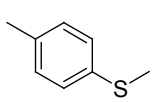
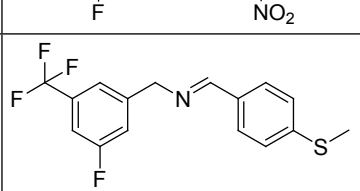
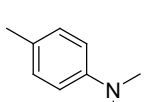
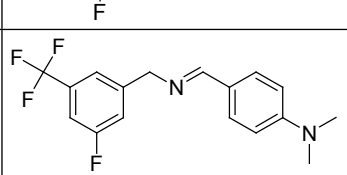
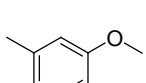
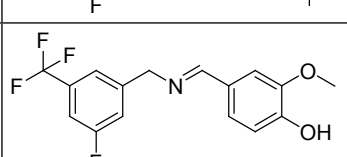
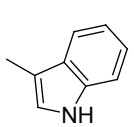
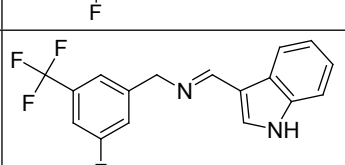
Compound	R	Structure	UV-visible (nm)	Yield (%)	Solubility
3a			235	75.60	Methanol
3b			240	71.23	Methanol
3c			245	74.59	Methanol
3d			265	66.29	Methanol
3e			260	64.98	Methanol
4f			250	80.99	Methanol
3g			260	67.21	Methanol
3h			270	68.78	Methanol
3i			240	68.81	Methanol

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Table 3 Antiproliferative activity of 3(a-i) against cancer cells determined by MTT test (µM)

Compound	% Cell survival								
	MCF7			U373			C6		
	100	200	400	100	200	400	100	200	400
3a	10.49	14.02	16.40	29.74	49.14	68.96	34.62	47.98	57.29
3b	12.31	17.45	26.56	31.49	45.78	67.56	36.25	48.71	59.91
3c	13.89	19.65	27.14	32.56	47.12	65.21	31.29	47.25	59.45
3d	29.01	30.05	31.48	30.03	32.33	37.93	28.26	35.96	40.63
3e	61.25	69.58	76.01	59.78	64.12	73.45	64.29	68.11	74.35
3f	27.65	38.21	51.63	30.58	42.87	57.56	29.51	45.31	60.19
3g	18.81	21.65	31.14	25.48	37.19	55.21	21.29	37.29	49.99
3h	15.49	18.56	29.49	30.54	41.14	61.16	26.19	45.25	55.88
3i	60.19	64.45	74.21	58.46	62.13	71.89	63.41	66.69	69.81
Control	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

REFERENCE

[1] Z. Cimerman, S. Miljanic and N. Galic, Croatica Chemica Acta, 73 (1), 81- 95 (2000). | [2] P. Singh, R. L. Goel and B. P. Singh, J. Indian Chem. Soc., 52, 958 (1975). | [3] B. F. Perry, A. E. Beezer, R. J. Miles, B. W. Smith, J. Miller and M. G. Nascimento, | Microbois., 45, 181 (1988). | [4] A. Elmali, M. Kabak and Y. Elerman, J. Mol. Struct., 477, 151 (2000). | [5] P. R. Patel, B. T. Thaker and S. Zele, Indian J. Chem., 38 A, 563 (1999). | [6] M. Valcarcel and M. D. Laque de Castro, "Flow-Throgh Biochemical Sensors", Elsevier, | Amsterdam (1994). | [7] U. Spichiger-Keller, "Chemical Sesors and Biosensors for Medical and Biological | Applications", Wiley-VCH, Weinheim (1998). | [8] J. F. Lawrence and R. W. Frei, "Chemical Derivatization in Chromatography", Elsevier, | Amsterdam (1976). | [9] M. A. Gawad, Y. M. Issa, S. M. Abd-Alhamid, Egypt. J. Pharm. Sci., 73, 55 (1996). | [10] N. Sari, S. Arslan, E. Logoglu, I. Sariyan, G. U. J. Sci., 16, 287, (2003). | [11] K.L. Kirk, Curr. Top. Med. Chem. 6, 1447-1456 (2006). | [12] P. Shah, A.D. Westwell, J. Enzym. Inhib. Med. Chem. 22, 527-540 (2007). | [13] J. Swinson, Pharm. Chem. 6, 38-41 (2007). | [14] C. Isanbor, D. O'Hagan, J. Fluorine Chem. 127, 303-319 (2006). | [15] M. Hudlicky, Chemistry of Organic Fluorine Compounds II, American Chemical Society, Washington, DC, 1, 979-1145 (995). | [16] G.D. Prestwich, Pesticide Science 17, 430-440 (1986) . | [17] B.K. Park, N.R. Kitterringham, P.M. Oneill, Annu. Rev. Pharmacol. Toxicol. 41, 443-470 (2001). | [18] C.C. Tang, Y.C. Li, B. Chen, H.Z. Yang, G.Y. Jin, Pesticide Chemistry. 1st ed., Tianjing China, 1998. | [19] C.D.S. Tomlin, The Pesticide Manual: A World Compendium, 13th ed., British Crop Protection Council: Alton, UK, 307-308 (2003). | [20] Mallesha L, Mohana KN, Veeresh B, Alvala R, Mallika A. Arch. Pharmacal Res.2012;35:51-57.