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Synthesis and spectroscopic characterization of fluorescent 4-aminoantipyrine analogues: Molecular docking and in vitro cytotoxicity studies

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ABSTRACT

Two substituted aromatic carbonyl compounds (compounds 1 and 2) of 4-aminoantipyrine were synthesized by condensation of fluorine substituted benzoyl chlorides and 4-aminoantipyrine. The structures of synthesized derivatives were established on the basis of UV–Vis, IR, and Mass, ^{1}H, ^{13}C NMR and Fluorescence spectroscopy. Both compounds showed significant fluorescence emission and two broad emission bands were observed in the region at 340 nm and 450 nm on excitation at 280 nm. Theoretically to prove that the molecule has anticancer activity against cervical cancer cells, the compounds were analyzed for molecular docking interactions with HPV16-E7 target protein by Glide protocol. Furthermore, 4-aminoantipyrine derivatives were evaluated for their in vitro cytotoxic activity against human cervical cancer cells (SiHa) by MTT assay. Compound 1 showed two fold higher activity (IC_{50} = 0.912 μM) over compound 2, and its activity was similar to that of Pazopanib, suggesting that although the two compounds were chemically very similar the difference in substituent on the phenyl moiety caused changes in properties.

1. Introduction

4-Aminoantipyrine and its derivatives are recognized for their variety of applications in the area of catalysis [1,2], medicine [3], and pharmacology [4]. Understanding the factors that promote drug innovation is important both for improvements in health care and for the future of organizations engaged in drug discovery research and development. New kinds of chemo-therapeutic agents containing Schiff bases have gained significant attention among the biochemists [1] and some of the aminopyrines are commonly administered intravenously to detect liver disease [3] in clinical treatment. New kinds of Schiff base compounds containing 4-aminoantipyrine have gained significant interest due to their potential applications in the area of catalysis [5–7] medicine [8] and pharmacology [9,10]. To probe the functional and application properties in chemotherapy diagnostic center a variety of 4-aminoantipyrine based agents are commonly administered intravenously to detect liver disease in clinical treatment [11]. Due to their significant roles in the environment, industrial and biological processes, research efforts have been devoted towards the design and development of simple and sensitive chromo sensors using 4-aminoantipyrine derivatives [12].4-Aminoantipyrine has been utilized as a key intermediate for the synthesis of heterocycles bearing biologically active moieties [13–15]. 4-Aminoantipyrine administration caused a significant decrease in the contracture of myometrium, while the cervix did not reveal significant change [16]. Binding studies of 4-aminoantipyrine with human serum albumin and DNA have already been explored [17,18]. 4-Aminoantipyrine derivatives are well recognized for their clinical and sensor abilities, however no attempt has been made to investigate their anticancer activity against cervical cancer. Furthermore, 4-aminoantipyrine aromatic acid derivatives have been studied less extensively with regard to anti-viral effects in viral infections that can subsequently lead to carcinomas. This study thus aims at assessing the effectiveness and plausible mode of action of compounds belonging to the class of 4AAP derivatives in HPV positive cervical cancer cells. As an effective receptor to design target based drug design, we selectively chose HPV16 E7 protein [18] and the designing and synthesizing of 4-aminoantipyrine derivatives became one of our major goals.

Towards this goal, this work reports the synthesis of two fluorescent 4-aminoantipyrine fluorine substituted benzoyl chloride derivatives, with a special focus on their structural investigation, molecular docking analysis with HPV16-E7 target protein receptor, and in vitro cytotoxicity against human cervical cancer cells (SiHa). Spectroscopic analyses explore the molecular structure and fluorescence of the compounds, shown in Fig. 1.

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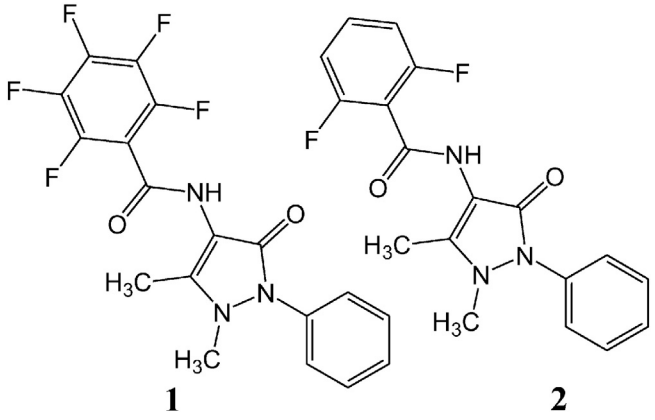


Fig. 1. Molecular structures of 4-aminoantipyridine derivatives 1 and 2.

2. Experimental section

2.1. Materials

4-Aminoantipyrine, 4-methylbenzoyl chloride, 3-methylbenzoyl chloride, and plasmid DNA were purchased from Aldrich & Co. SiHa cervical cancer cell line was purchased from NCCS, Pune INDIA. All cells were grown in 10% DMEM at 37 °C in 5% CO_2. Commercially available MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) and Eagle's minimal essential medium (EMEM) were obtained from Sigma (St Louis, MO). Caspase-3 apoptosis kit was purchased from BD Pharmingen, USA. All other reagents and solvents were of analytical grade.

2.2. Synthesis

2.2.1. Synthesis of compound 1

1:1 equimolar methanolic solution of 4-aminoantipyrine (0.203 g, 0.001 mol) and 1,2,3,4,5-pentafluorobenzoyl chloride (0.230 g, 0.001 mol) were mixed and refluxed for 90 min with constant stirring. The pale yellow precipitate obtained by condensation was filtered out and recrystallized in methanol.

N-(1, 5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-2,3,4,5,6-pentafluoro-benzamide. NMR data (δ, *DMSO-D_6*): 10.19 (*s*, *–NH*, *1H*), 7.53–7.49 (t, *phenyl*, *2H*), 7.37–7.32 (*q*, *phenyl*, *3H*), 7.21–7.17 (*tr*, *Ar*, *2H*), 3.11 (*s*, *N–CH_3*, *3H*), 2.20 (*s*, *C–CH_3*, *3H*). ^{13}C-NMR: (δ, *DMSO-D_6*) 160 (C═O, amide), 155.95 (C═O), 135.69 134.75, 129.07, 126.82, 126.502, 124.00, 123.83, 103.83, 105.65 (aryl carbons) 105 (NH–C═) 35.71 (N–Me), 11.04 (C–Me). IR data (KBr, cm^{-1}): 3140, 2937, 1950, 1800, 1697, 1633, 1514, 1425, 1365, 1321, 1240, 1133, 1100, 991, 962, 854, 804, 258, 700, 594. UV–Vis [DMSO,λ_{max},nm,], 263, 292. ES [MSI] calculated for $C_{18}H_{12}F_5N_3O_2$ (m/z) = 397.30, found = 397.13.

2.2.1.1. Analytical data. Calculated for $C_{18}H_{12}F_5N_3O_2$. Calc: C, 54.42; H, 3.04; N, 10.58%. Found: C, 54.22; H, 3.09; N, 10.65%.

2.2.2. Synthesis of compound 2

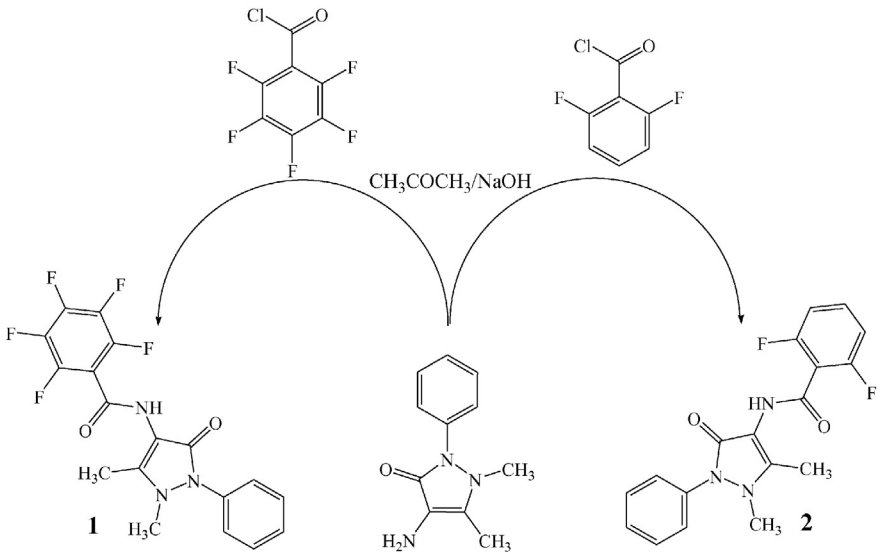
1:1 equimolar methanolic solution of 4-aminoantipyrine (0.203 g, 0.001 mol) and 2, 6-Difluorobenzoyl chloride (0.176 g, 0.001 mol) were mixed and refluxed for 90 min with constant stirring. The pale yellow precipitate obtained by condensation was filtered out and recrystallized in methanol.

N-(1, 5-dimethyl-3-oxo-2-phenyl-2, 3-dihydro-1H-pyrazol-4-yl)-2, 6-difluoro-benzamide. The same synthetic procedure adopted for compound 1 was repeated for compound 2, appropriately adapting 2, 6-difluorobenzoyl chloride in place of 1,2,3,4,5-pentafluorobenzoyl. NMR data (δ, *DMSO-D_6*): 9.91 (*s*, *–NH*, *1H*), 7.56–7.49 (*q*, *phenyl*, *3H*), 7.37–7.31 (*q*, *phenyl*, *3H*), 7.21–7.17 (*tr*, *phenyl*, *2H*), 3.10 (*s*, *N–CH_3*, *3H*), 2.20 (*s*, *C–CH_3*, *3H*). ^{13}C-NMR: (δ, *DMSO-D_6*) 160.07 (C═O, amide), 159.12 (C═O), 152.27, 134.86, 131.75, 131.95, 129.09, 126.45, 123.77, 112.00, 111.95, 111.76, 106.27 (*aryl carbons*) 106.27 (NH–C═) 35.82 (N–Me), 11.04 (C–Me), IR data (KBr, cm^{-1}) 3508, 3444, 1662, 1625 1496, 1457, 1413, 1348, 1333, 1236, 1010, 906, 852, 796, 608, 530. UV–Vis [DMSO, λ_{max}, nm,], 260, 290. ES (MSI): Calculated for $C_{18}H_{15}F_2N_3O_2$ (m/z) = 343.33; Found = 343.15.

2.2.2.1. Anal data. Calculated for $C_{18}H_{15}F_2N_3O_2$. Calc: C, 62.97; H, 4.40; N, 12.24%. Found: 62.68; H, 4.54; N, 12.07%.

2.3. Physical measurements

Microanalysis of the complexes was done using a PerkinElmer PE 2400 series II CHNS/O elemental analyzer. Electronic spectra were recorded on a Jasco 670 spectrophotometer. Fluorescence spectra were recorded on a Jasco 8300 spectrophotometer. ^{1}H NMR spectroscopy in DMSO-d6 (500 MHz, Bruker), ^{13}C NMR spectroscopy in DMSO-d6 (125 MHz, Bruker) using tetramethylsilane (TMS) as internal standard



Scheme 1. Synthesis of 4-aminoantipyridine derivatives 1 and 2.

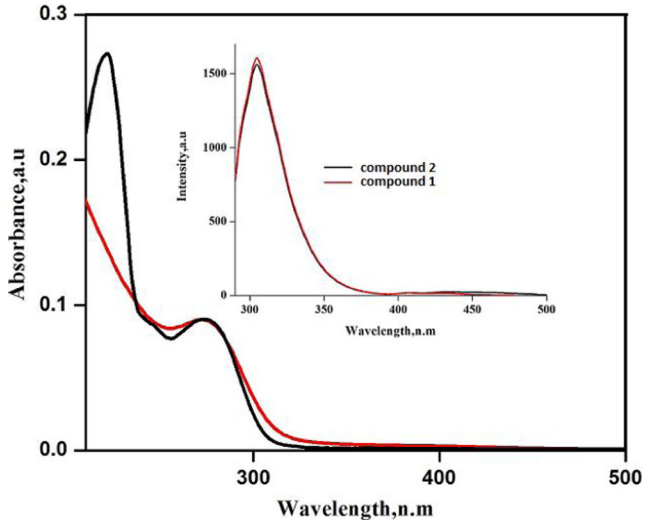


Fig. 2. UV–Visible spectrum of compounds 1 and 2 in ethanol (inset: fluorescence emission spectrum of compound 1 and 2 excited at 290 nm).

were also carried out. Chemical shifts for proton resonances are reported in ppm (δ) relative to tetramethylesilane and *DMSO-D_6* was used as solvent. High-resolution mass spectra (HRMS-EI) was measured by Electron ionization (EI) method (Jeol GC-Mate 2). Molecular docking studies of all the synthesized compounds were accomplished by GLIDE program (version 8.5, Schrodinger, LLC, New York, 2010) and the entire glide scores are reported in kcal/mol. In vitro cytotoxicity of all the compounds against cervical cancer cell line (SiHa) was studied by cell viability assay method.

2.4. Molecular docking studies

Molecular docking has become an increasingly important tool for drug discovery. It is a useful tool for investigating the binding interaction of a protein receptor with its ligand and detail about the binding mechanism. Flexible ligand with rigid receptor docking strategies were employed in this study to identify the most suitable ligand binging sites in the compounds 1 and 2 with human papilloma virus type 16 E7 protein [19,20]. To prove the biological activity through computational method the protein molecule should have the three dimensional molecular characterization and in view of the study the three dimensional structure of E7 protein was predicted and downloaded through Robetta server. Molecular docking studies were performed using Schrödinger Glide program (version version 2014-4, Schrödinger LLC, New York 2014) [21]. To perceive the docking glide score results Maestro user interface was executed and to confirm the best docking and validate the docking score, the protocol was evaluated by re-docking. To compare the molecular interaction with the standard reference drug, Pazopanib [22] was selected for docking studies. The structures of compounds 1, 2, and the reference molecule were drawn using ACD/Chemsketch (free ware). The Glide Grid generation wizard was used to define the grid space to reveal the biological active site. Glide docking was accomplished using SP (Single precision) and XP (Extra precision) docking procedure.

2.5. Biological assay

2.5.1. In vitro cytotoxicity analysis

In vitro cytotoxicity analysis was performed using standard procedures available in previous reports [23–27]. SiHa cervical cancer cell line was analyzed for contaminants. The contaminant free SiHa cells was cultured in complete EMEM growth medium (EMEM + 10% FBS). 90% confluent SiHa mono layer culture was trypsinized and was diluted with complete growth medium. 10,000 cells were placed in each well of a 96-well plate, and the plate was incubated in a CO_2 incubator for 24 h at 37 °C. The medium was then aspirated out and 95 μl of fresh medium and 5 μl of drug was added. The plate was incubated in CO_2 incubator for 48 h at 37 °C. 20 μl medium was removed and replaced with 20 μl MTT. The plate was incubated in CO_2 incubator for 4 to 6 h at 37 °C. The entire medium was carefully removed and the formazan crystals were dissolved in DMSO. The plate was read at 570 nm. The % growth inhibition was calculated by % cell inhibition = 100 − ({(At − Ab) / (Ac − Ab)} × 100), where, At − absorbance value of test compound. Ab − absorbance value of blank, and Ac − absorbance value of control.

3. Results and discussion

3.1. Synthesis

The 4-antiaminopyrine derivatives 1 and 2, possessing fluorine substituted phenyl moiety, were synthesized by acid chloride and amine condensation reaction in acetone at 40 °C as represented in Scheme 1.

The compounds 1 and 2 are pale yellow in color and are soluble in almost all organic solvents, such as acetone, methanol, acetonitrile, chloroform, DMF, and DMSO.

3.2. Spectral investigation

3.2.1. UV–Vis spectroscopy

The UV–Visible spectrum for compounds 1 and 2 were recorded in ethanol. Both compounds exhibited similar absorption spectra (Fig. 2).with two bands, one at 260 nm (π–π* transition) and the other at 290 nm (n–π* transition). The later is due to non-bonding electrons present on the nitrogen atom of the CO group [28].

3.2.2. Fluorescence spectra

Fluorescence emission spectra of compounds 1 and 2 were recorded in 10^{-5} M solution of ethanol at room temperature (Fig. 2). Emission band observed region at 320 nm when excited at 280 nm. Both compounds behave similarly due to the 4-aminoantipyrine core which is common.

3.2.3. IR spectra

FT-IR spectra of compounds 1 and 2 were recorded to identify functional groups [Supplementary material S1-1]. The three characteristic IR signals for compounds 1 and 2 corresponding to (i) CO, (ii) NH, and

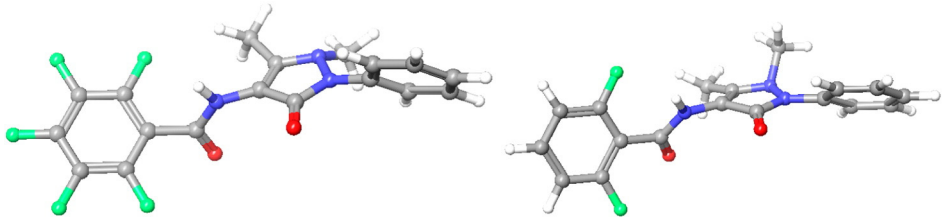


Fig. 3. Optimized geometry of compounds 1 and 2.

(iii) C–N were analyzed. The broad IR signal around 3800–3000 cm^{-1} obtained for both compounds 1 and 2 can be ascribed to the NH functional groups. Compounds 1 & 2 showing sharp and intense peaks in the range of 1572–1589 cm^{-1} and 1616–1665 cm^{-1} can be attributed to ν_{C-N} and $\nu_{C=O}$ stretching modes respectively.

3.2.4. Mass spectra

The mass spectral data [Supplementary material SI-2, 3] indicating an intense molecular ion peak at 343.17 and 394.13 corresponds to respective (M + 1) molecular formulae, confirming the formation of compounds 1 and 2 respectively.

3.2.5. NMR spectra

^{1}H and ^{13}C-NMR spectroscopic techniques were carried out and the compounds were characterized without any ambiguity as described below. The ^{1}H NMR spectra recorded on DMSO-D_6, (SI-4) independently provides well resolved ^{1}H NMR signals attributable to aliphatic protons N–CH_3 and C–CH_3, in addition to aromatic phenyl protons, and NH resonances and the integral area matches with the calculated number of hydrogens appropriately.

The ^{13}C NMR spectra (SI-5) recorded in $CDCl_3$ provides many well distinguishable peaks confirming the presence of various magnetically nonequivalent carbons. The peaks around 160 and 155δ indicate the presence of two different C═O groups attributed to antipyrine CO and peptide CO. In the aromatic region, the peak corresponding to 131.37 and 131.57δ with a weak intensity represents the quaternary carbon. The other peaks observed at 129.83, 128.58, 128.49δ corresponds to the aromatic CH carbons. The peak at 70.49δ indicates the presence of –CH_2, the ^{13}C resonance at 35δ and 10δ represents the N–CH_3 and C–CH_3 carbons respectivly.

3.2.6. Molecular docking studies

The optimized geometry of compounds 1 and 2 are shown in Fig. 3. Both structures are almost similar. The fluorine substituted phenyl ring is situated nearly perpendicular to the 4-aminoantipyrene ring.

To comprehend the molecular interaction of HPV16 E7 protein receptor with chemically synthesized molecules of compounds 1 and 2, the three dimensional structure of HPV16 E7 protein was predicted and downloaded from Robetta server [29] and deliberated with Schrodinger GLIDE program. All Glide Docking and Emodel scores were analyzed and the details are presented in Table 1. Aromatic carbonyl functional groups of both compounds were established. Before docking, compounds 1 and 2 were optimized using Schrödinger software to have proper atomic coordination. Docking studies revealed intermolecular hydrogen bonding interaction with amino hydrogens of GLN70 of receptor protein and amide and antipyrine carbonyl groups of compounds 1 and 2. The standard reference drug pazopanib interacted through hydrogen bonding with GLN70, CYS68, ASP14, and ASP4 (Fig. 4). All the Glide and Emodel scores were compared with the scores of the standard drug. Computational analysis of the compounds clearly reveal significant relationship of 4-aminoantipyrine fluorinated benzoyl derivative with the HPV16 E7 receptor moieties, suggesting that increasing the number of fluorine in benzoyl moiety may increase the efficacy of the drug.

Table 1
Molecular docking data of compounds 1, 2 and standard reference drug (Pazopanib) with HPV16 E7 protein receptor.

Compounds	Molecular docking			
	Glide score (kcal/mol)	Emodel score	Binding Energy	XP hydrogen bond
1.	−12.658	−58.79	−49.612	2 (GLN 70)
2.	−12.073	−76.849	−64.731	2 (GLN 70)
3.	−11.356	−38.717	−532.487	4 (GLN70, CYS 68, ASP14, ASP4)

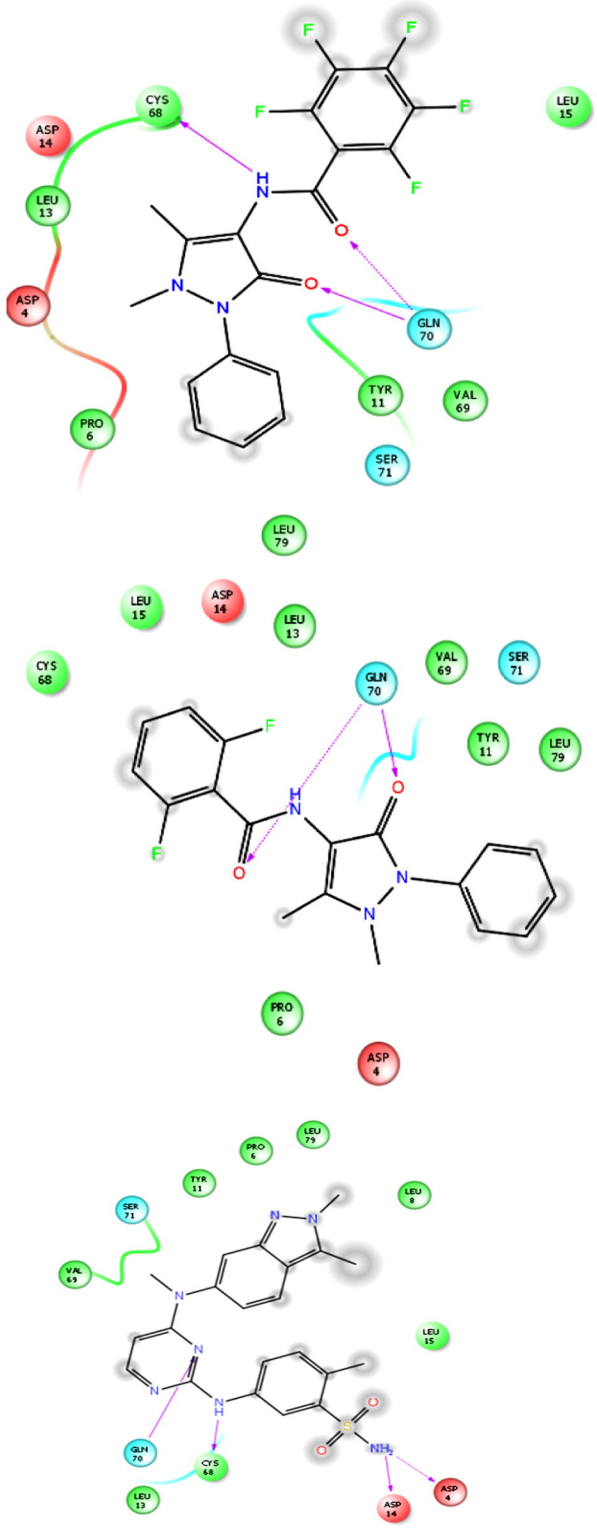


Fig. 4. Docking model structure of compounds 1, 2 and reference drug (Pazopanib) into the HPV16 E7 Protein Binding Pocket.

3.2.7. In vitro cytotoxicity assay

In vitro cytotoxicity was estimated using MTT assay. Results revealed the anticancer efficacy of compounds 1 and 2 on human cervical cancer cells (Fig. 5). Morphological alteration was obtained in all the tested concentrations and the two compounds showed dose dependent anticancer activity (Fig. 6). Maximum anticancer activities for both compounds were observed at 100 μM. The percentage of cell growth

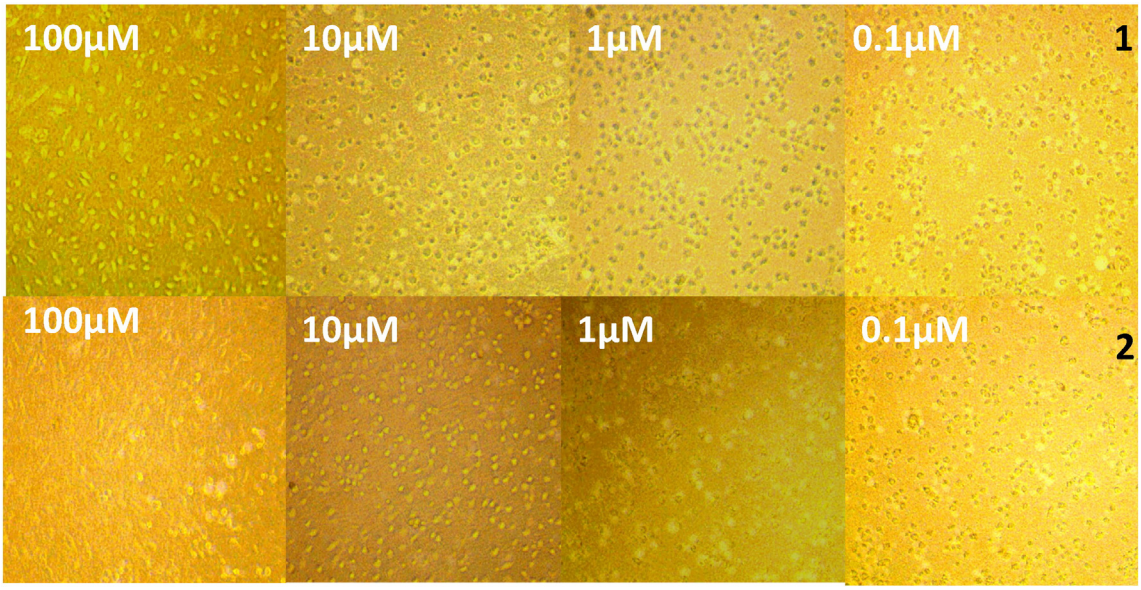


Fig. 5. Images of cytotoxic activity of compound 1 and 2 in SiHa cell line.

inhibition was found to be 93.02% for compound 1 and 85.27% for compound 2. The IC50 values were estimated from Fig. 7, and was found to be 0.912 µM for compound 1 and 1.02 µM for compound 2 and 0.8 µM for Pazopanib, revealing that the potency of compound 1 was closer to standard than compound 2. The higher anticancer potency of compound 1 is probably due to the presence of high number of fluorine in benzoyl moiety in the 4-aminoantipyrine. This might be the reason for the notable difference in IC50 values of the synthesized 4-aminoantipyrine derivatives (Fig. 8).

4. Conclusion

In conclusion, a new series of fluorine substituted fluorescent 4-aminoantipyrine derivatives were chosen through target based drug discovery, and synthesized, and characterized by UV–Visible, FT-IR, ^{1}H NMR, ^{13}C NMR and high resolution mass (HRMS-EI) spectroscopy. Compounds 1 and 2 showed significant fluorescence emission, two broad emission bands were observed in the region of 340 nm and 450 nm on excitation at 280 nm. Molecular docking studies establish that these molecules exhibit significant molecular interactions with HPV16-E7 target protein that are responsible for causing cervical cancer in human. In vitro cytotoxicity study of the synthesized molecules against cervical cancer cells (SiHa), revealed that compound 1 exhibited better IC50 value (0.912 µM) than compound 2 (1.02 µM) and was similar to that of the standard drug. Both compounds inhibited the growth of SiHa cells, with compound 1 showing a cell growth inhibition of 93.02% and compound 2 showing a cell growth inhibition of 85.27%. On comparing the results of Glide score and in vitro cytotoxicity studies, it is clear that these two fluorine substituted fluorescent 4-aminoantipyrine derivatives are moderately effective in inhibiting the growth of cancer cells (SiHa). The difference in fluorine substitution on the phenyl moiety exhibited changes in anticancer potency of the compounds, even though both these compounds were derived from a common structure.

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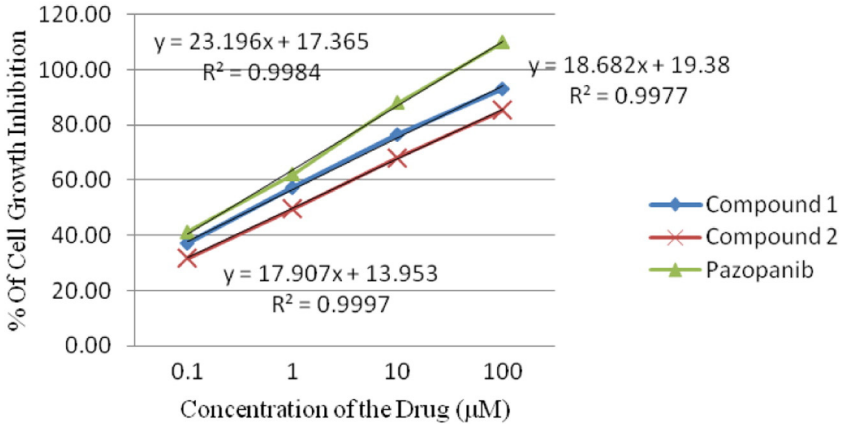

Fig. 6. Determination of IC50 value of the compound by MTT assay.

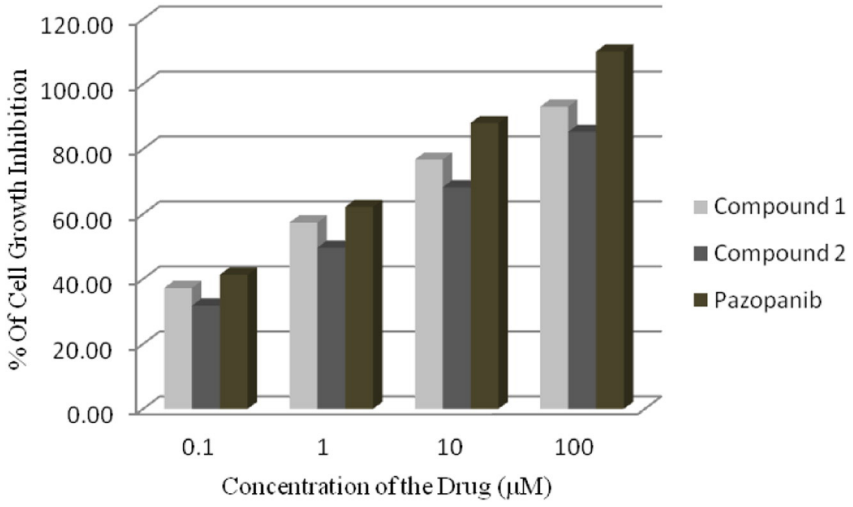

Fig. 7. Anticancer effect of compounds 1 and 2 on SiHa cell line.

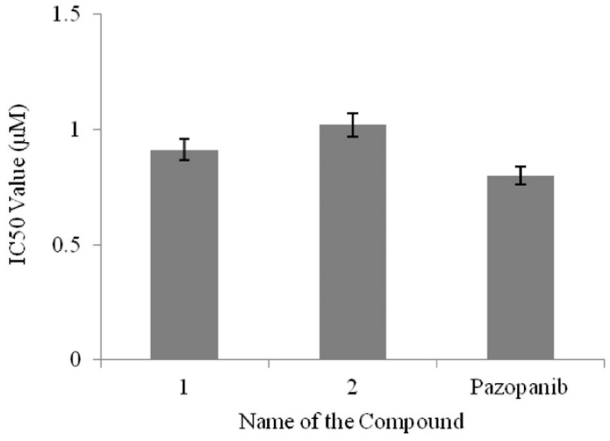

Fig. 8. Analysis of IC50 values of compounds 1 and 2 on SiHa cells.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.saa.2015.08.008.

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