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Synthesis and Biological Evaluation of Theophylline Methyl 1,3,4-Oxadiazole as Anticancer Agents

Vindya K. Gopinatha[a], Ujjayinee Ray[b], Kempegowda Mantelingu[a], Sathees C. Raghavan[b], and Kanchugarakoppal S. Rangappa[a, 1]

[a] *Department of Studies in Chemistry, Manasagangotri, University of Mysore, Mysuru, 570006 India*

[b] *Department of Biochemistry, Indian Institute of Science, Bangalore, 560012 India*

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Abstract—A series of theophylline methyl 1,3,4-oxadiazole small molecules were obtained via cyclization of theophylline-7-acetohydrazide with different benzoic acids. The compounds (**IVa–j**) were synthesized and characterized by using conventional methods. The new compounds obtained were evaluated for their cytotoxic effect in three different cancer lines, the activity obtained varies depending upon the structure of a molecule. The compound (**IVb**) and (**IVf**) showed promising effect than other compounds of the series. Thus, these two derivatives have the potential for developing as anticancer agents.

Keywords: theophylline, cytotoxicity, oxadiazole, cancer

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INTRODUCTION

Cancer is a disease characterized by uncontrolled cell growth, finding an effective treatment for cancer is one of the major global challenge. Development of novel small molecule inhibitors against cancers is an area of active research [1]. The five-member heterocyclic compounds, particularly nitrogen and oxygen heterocycles are commonly used scaffolds. 1,3,4-Oxadiazoles are non-naturally occurring five-membered aromatic heterocycles received special attention in pharmaceutical chemistry due to their diverse medicinal potential [2, 3]. They have demonstrated a broad spectrum of biological activities such as anticancer [4], antituberculosis [5, 6], antifungal [7], antiviral [8], antimicrobial [9], anticonvulsant [10], anti-inflammatory and analgesic [11, 12].

Considering the importance of this scaffold, we focused our research on the synthesis and characterization of novel theophylline methyl 1,3,4-oxadiazole derivatives with different substituents on the phenyl ring. Moderate to excellent yields of theophylline methyl 1,3,4-oxadiazole derivatives were obtained and investigated for their potential as anticancer agents. Of these, two derivatives (**IVb**) and (**IVf**) showed good efficacy in killing cancer cells at concentrations as low as 28 μM.

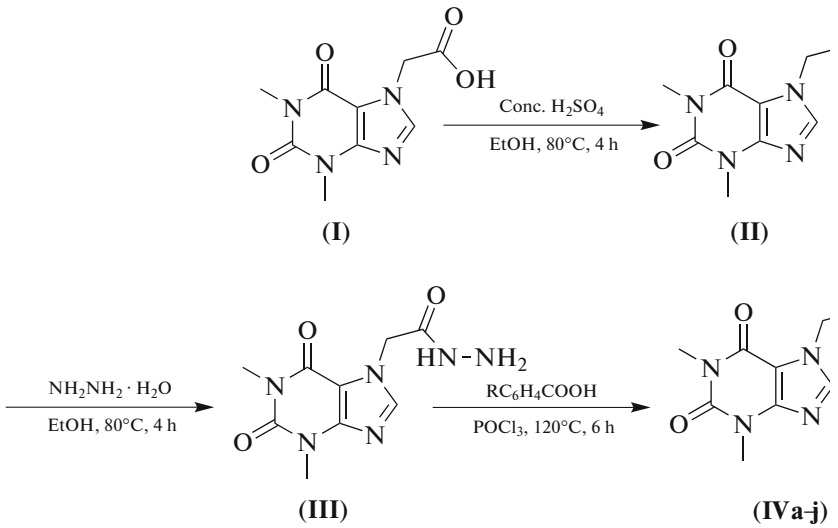
RESULTS AND DISCUSSION

Chemistry

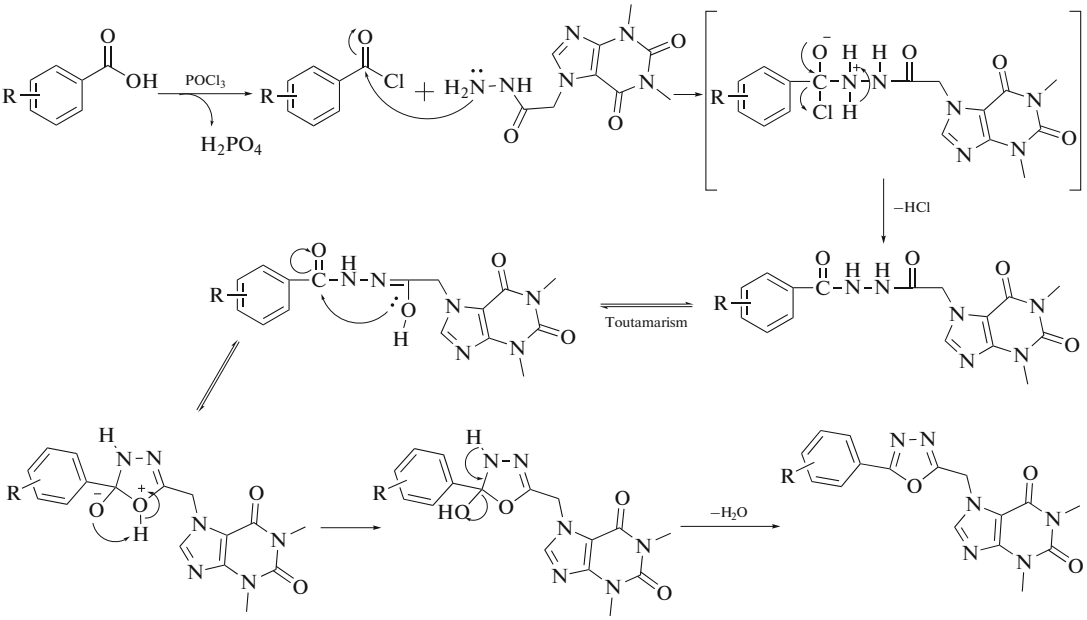
In continuation of our work on the synthesis of bioactive heterocyclic compounds [13–15], the series of novel theophylline methyl 1,3,4-oxadiazole derivatives, which possess different substituents on the phenyl ring were synthesized according to Scheme 1. When theophylline acetic acid was treated with ethanol in the presence of H_2SO_4 provided the compound (**II**) in a quantitative yield, the compound (**II**) in turn reacted with hydrazine hydrate and resulted in a very good yield of theophylline acetohydrazide (**III**). Lastly, the cyclization process of hydrazide was carried out with different benzoic acids in the presence of $POCl_3$ which resulted oxadiazole derivatives in moderate to excellent yields. The accomplishment of the cyclization process resulted in the formation of the theophylline methyl 1,3,4-oxadiazole. A probable mechanism for the formation of oxadiazole compound is shown in Scheme 2 [16].

Structure confirmation of all synthesized compounds was done by spectroscopic techniques, such as ^{1}H NMR, ^{13}C NMR and LC-MS, results are summarized in the experimental section. All molecules showed relatively similar spectroscopic data, in ^{1}H NMR spectra, the protons from the one of ($N-CH_3$) group was recorded as a singlet between 3.20–3.30 while the other appears between 3.40–3.50 ppm, the protons of the methylene group (CH_2-N) resonates as singlet between 5.80–6.00 ppm and the remaining aromatic protons appeared between 7.5–8.5 ppm. Finally, ^{13}C NMR spectra provided the final structural elucidation of the derivatives. The mass spectra of all compounds exhibit well-defined molecular ions.

[1] Corresponding author: e-mail: rangappaks@gmail.com.



Scheme 1. Synthesis of theophylline methyl 1,3,4-oxadiazole derivatives (**IVa–j**).



Scheme 2. Plausible reaction mechanism for the formation of (**IVa–j**).

BIOLOGY

In the present study, we have investigated the therapeutic potential of 10 novel oxadiazole derivatives (**IVa–j**). Cytotoxicity of the derivatives were evaluated in three different cancer cell lines, HeLa, CEM and Nalm6. The results were compared to that of SCR7, a previously reported small molecule inhibitor showing anticancer potential [17, 18]. IC_{50} of the compounds were plotted in tabular form (Table 1). In adherent cell line HeLa, (**IVd**) showed the best cytotoxicity with IC_{50} around 28 μM (Fig. 1, Table 1). This was closely followed by (**IVf**), (**IVj**), (**IVc**), (**IVi**) and (**IVb**) with observable 50% inhibitory effect on cells at concentrations between 50 and 73 μM. In human T cell leukemic cell line CEM, most of the compounds have high IC_{50}, although (**IVf**) and (**IVb**) showed promising cytotoxicity at around 28 and 40 μM, respectively (Fig. 2, Table 1). In B cell precursor leukemia cell line Nalm6, (**IVf**) and (**IVb**) showed significantly good effect on cell death compared to other compounds (Fig. 3, Table 1). IC_{50} for (**IVb**) and (**IVf**) were obtained at around 65 and 47 μM.

EXPERIMENTAL

Chemistry

Chemicals and reagents. The chemicals used were obtained from Sigma-Aldrich (USA). The reaction progress was monitored by TLC, which was performed

on Merck silica gel 60 F_{254} plates and visualization was accomplished under UV light. Melting points were measured using open capillary method and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded by Agilent WM Fourier transforms spectrophotometer and Bruker operating at 400 and 100 MHz respectively, using DMSO-d_6 as a solvent and tetramethylsilane (TMS) as internal standard. High Resolution Mass Spectra was recorded on a Waters SYNAPT G2 instrument.

General procedure for the synthesis of theophylline theophylline methyl 1,3,4-oxadiazole (IVa–j). To a mixture of substituted benzoic acid (0.01 mol) and theophylline acetohydrazide (**III**) (0.01 mol), 2 mL of $POCl_3$ was added. The reaction mixture was refluxed for 6–8 h, after completion of the reaction, the contents were cooled to room temperature and poured into crushed ice, then the mixture was neutralized with 20% solution of $NaHCO_3$ to obtain solid, further the solid was dried and purified by column chromatography employing 24 : 1 of CH_2Cl_2 : CH_3OH as eluent.

1,3-Dimethyl-7-((5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl)methyl)-1H-purine-2,6(3H,7H)-dione (IV a). Off white solid; R_f: 0.3; Yield: 90%; Melting Point: 252–254°C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 8.45 (d, J = 8.8 Hz, 2H), 8.33 (s, 1H), 8.26 (d, J = 8.8 Hz, 2H), 6.01 (s, 2H), 3.48 (s, 3H), 3.21 (s, 3H); ^{13}C NMR (DMSO-d_6, 400 MHz): δ = 163.1, 163.0, 154.3, 150.9, 149.3, 148.2, 143.3, 128.4, 127.9, 124.6, 106.0, 40.8, 29.4, 27.4; HRMS (ESI): (m/z) $[M + H]^+$ calcd. for $C_{16}H_{13}N_7O_5$: 384.1012, found 384.1010.

7-((5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl)-methyl)-1,3-dimethyl-1H-purine-2,6(3H,7H)-dione (IVb). Off white solid; R_f: 0.4; Yield: 85%; Melting Point: 278–280°C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 8.31 (s, 1H), 8.01–8.11 (m, 2H), 7.67–7.74 (m, 1H), 7.44–7.49 (m, 1H), 5.95 (s, 2H), 3.47 (s, 3H), 3.20 (s, 3H); ^{13}C NMR (DMSO-d_6, 400 MHz): δ = 163.7, 162.9, 154.3, 150.9, 148.2, 143.2, 129.3, 129.2, 124.3, 119.1, 106.0, 40.7, 29.4, 27.4; HRMS (ESI): (m/z) $[M + H]^+$ calcd. for $C_{16}H_{13}ClN_6O_3$: 373.0738, found 373.0729.

1,3-Dimethyl-7-((5-phenyl-1,3,4-oxadiazol-2-yl)-methyl)-1H-purine-2,6(3H,7H)-dione (IVc). Off white solid; R_f: 0.4; Yield: 82%; Melting Point: 262–264°C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 8.33 (s, 1H), 7.99 (dd, J = 8.2 Hz, 1.4 Hz, 2H), 7.62–7.66 (m, 3H), 5.96 (s, 2H), 3.48 (s, 3H), 3.21 (s, 3H); ^{13}C NMR (DMSO-d_6, 400 MHz): δ = 164.4, 162.2, 154.3, 150.9, 148.3, 143.2, 132.2, 129.4, 126.5, 122.9, 106.1, 40.8, 29.4, 27.4; HRMS (ESI): (m/z) $[M + H]^+$ calcd. for $C_{16}H_{14}N_6O_3$: 339.1161, found 339.1164.

1,3-Dimethyl-7-((5-(3-nitrophenyl)-1,3,4-oxadiazol-2-yl)methyl)-1H-purine-2,6(3H,7H)-dione (IVd). Off white solid; R_f: 0.3; Yield: 88%; Melting Point: 266–268°C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 8.66–8.67 (m, 1H), 8.48–8.51(m, 1H), 8.41–8.43 (m, 1H), 8.34 (s, 1H), 6.00 (s, 2H), 3.48 (s, 3H), 3.21 (s, 3H); ^{13}C NMR (DMSO-d_6, 400 MHz): δ = 163.0, 162.9, 154.3, 150.9, 148.3, 148.2, 143.3, 132.5, 131.4, 126.5, 124.3, 121.0, 106.0, 40.8, 29.4, 27.4; HRMS (ESI): (m/z) $[M + H]^+$ calcd. for $C_{16}H_{13}N_7O_5$: 384.1012, found 384,1015.

7-((5-(4-Methoxyphenyl)-1,3,4-oxadiazol-2-yl)-methyl)-1,3-dimethyl-1H-purine-2,6(3H,7H)-dione (IVe). Off white solid; R_f: 0.4; Yield: 82%; Melting Point: 270–272°C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 8.32 (s, 1H), 7.92 (dd, J = 6.8 Hz, 2 Hz, 2H), 7.14–7.18 (m, 2H), 5.93 (s, 2H), 3.87 (s, 3H), 3.48 (s, 3H), 3.21 (s, 3H); ^{13}C NMR (DMSO-d_6, 400 MHz): δ = 164.3, 162.1, 161.6, 154.3, 148.3, 143.2, 128.4, 115.2, 114.9, 106.0, 55.5, 40.8, 29.4, 27.4; HRMS (ESI): (m/z) $[M + H]^+$ calcd. for $C_{17}H_{16}N_6O_4$: 369.1261, found 369.1255.

7-((5-(2-Bromophenyl)-1,3,4-oxadiazol-2-yl)-methyl)-1,3-dimethyl-1H-purine-2,6(3H,7H)-dione (IVf). Off white solid; R_f: 0.4; Yield: 86%; Melting Point: 288–290°C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 8.31 (s, 1H), 7.90 (dd, J = 7.4 Hz, 1.8 Hz, 2H), 7.55–7.64 (m, 2H), 5.99 (s, 2H), 3.47 (s, 3H), 3.21 (s, 3H); ^{13}C NMR (DMSO-d_6, 400 MHz): δ = 163.2, 162.6, 154.3, 150.9, 148.2, 143.3, 134.3,133.5, 131.7, 128.3, 124.2, 120.7, 106.1, 40.8, 29.5, 27.4; HRMS (ESI): (m/z) $[M + H]^+$ calcd. for $C_{16}H_{13}BrN_6O_3$: 418.0212, found 418.0210.

7-((5-(4-Bromophenyl)-1,3,4-oxadiazol-2-yl)methyl)-1,3-dimethyl-1H-purine-2,6(3H,7H)-dione (IVg). Off white solid; R_f: 0.4; Yield: 84%; Melting Point: 283–285°C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 8.30 (s, 1H), 7.73 (d, J = 8.4 Hz, 2H), 7.48 (d, J = 8.8 Hz, 2H), 5.95 (s, 2H), 3.43 (s, 3H), 3.20 (s, 3H); ^{13}C NMR (DMSO-d_6, 400 MHz): δ = 163.4, 162.3, 154.2, 150.1, 148.2, 143.1, 129.3, 129.2, 124.0, 118.8, 106.0, 40.7, 29.3, 27.3; HRMS (ESI): (m/z) $[M + H]^+$ calcd. for $C_{16}H_{13}BrN_6O_3$: 418.0212, found 418.0216.

7-((5-(4-Fluorophenyl)-1,3,4-oxadiazol-2-yl)methyl)-1,3-dimethyl-1H-purine-2,6(3H,7H)-dione (IVh). Off white solid; R_f: 0.4; Yield: 85%; Melting Point: 284–286°C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 8.28 (d, J = 8.8 Hz, 2H), 8.04 (s, 1H), 7.71(d, J = 8.8 Hz, 2H), 5.92 (s, 2H), 3.43 (s, 3H), 3.15 (s, 3H); ^{13}C NMR (DMSO-d_6, 400 MHz): δ = 163.7, 162.4, 154.4, 150.9, 148.2, 143.2, 129.6, 129.3, 126.8, 121.8, 106.0, 40.8, 29.4, 27.3; HRMS (ESI): (m/z) $[M + H]^+$ calcd. for $C_{16}H_{13}FN_6O_3$: 357.1067, found 357.1062.

7-((5-(2-Chlorophenyl)-1,3,4-oxadiazol-2-yl)methyl)-1,3-dimethyl-1H-purine-2,6(3H,7H)-dione (IVi). Off white solid; R_f: 0.4; Yield: 86%; Melting Point: 292–294°C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 8.27 (s, 1H), 7.94–7.98 (m, 1H), 7.65–7.70 (m, 1H), 7.40–7.43 (m, 2H), 6.00 (s, 2H), 3.43 (s, 3H), 3.18 (s, 3H);

Table 1. IC_{50} values of (**IVa–j**) in three different cancer cell lines. % of viable cells treated with individual compounds in HeLa, CEM and Nalm6 cell lines, 50% inhibitory concentration of each were calculated in micromolar

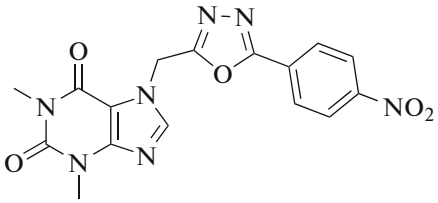
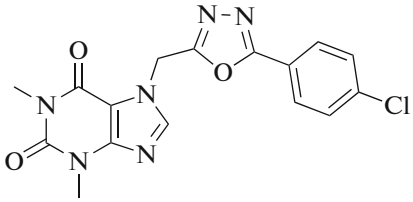
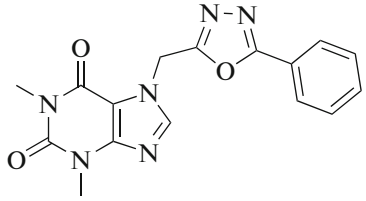
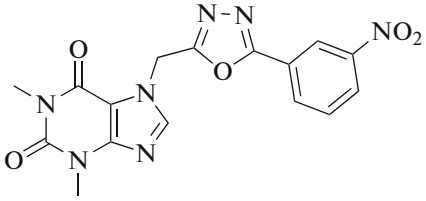
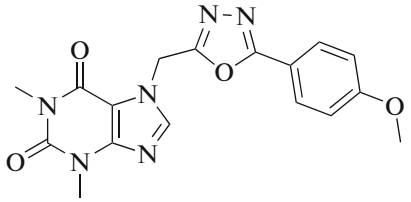
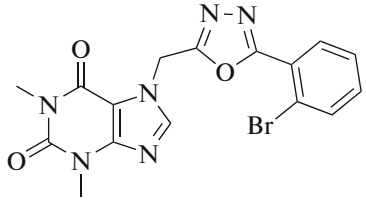
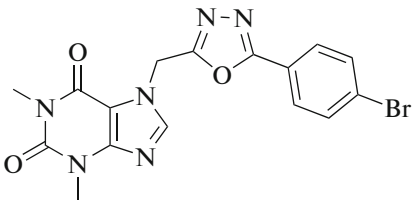
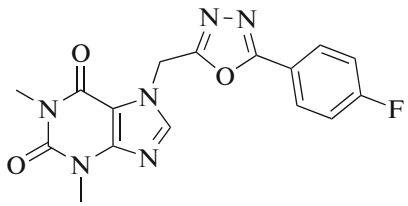
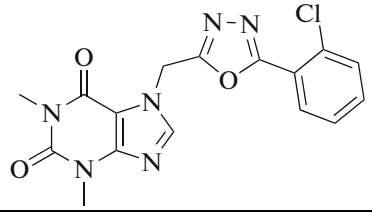
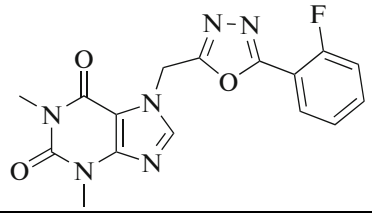
Entry	Structure	IC_{50}, μM		
		HeLa	CEM	Nalm6
(IVa)		121.77	>200	90.54
(IVb)		73.15	40.03	65.55
(IVc)		64.11	149.9	108.02
(IVd)		28.2	>200	>250
(IVe)		>200	>250	160.75
(IVf)		50	28.79	47.79
(IVg)		>200	>200	>200

Table 1. (Contd.)

Entry	Structure	IC_{50}, μM		
		HeLa	CEM	Nalm6
(IVh)		123.66	>250	140.68
(IVi)		72.24	>250	150.87
(IVj)		55.08	>200	140.47
SCR7		>50	>250	>50

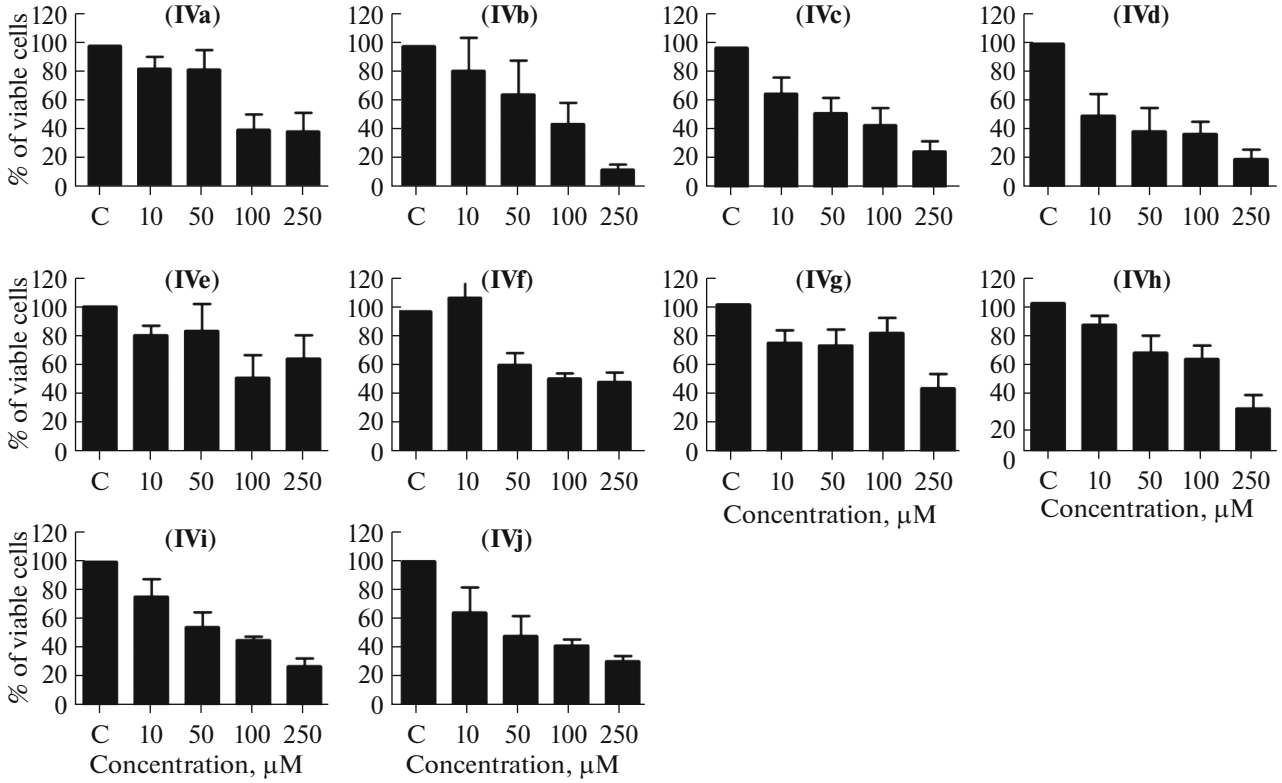


Fig. 1. Analysis of cytotoxicity of (**IVa–j**) in cervical cancer cell line, HeLa. Bar graph depicting cytotoxicity of (**IVa–j**) in HeLa cell line at increasing concentrations of compounds (10, 50, 100 and 250 μM). DMSO treated cells served as vehicle control. Bar graphs represent % of viable cells following treatment (48 h) with compounds. Experiments were performed at least three times and plotted using GraphPad Prism software. Bar graphs represent mean ± SEM.

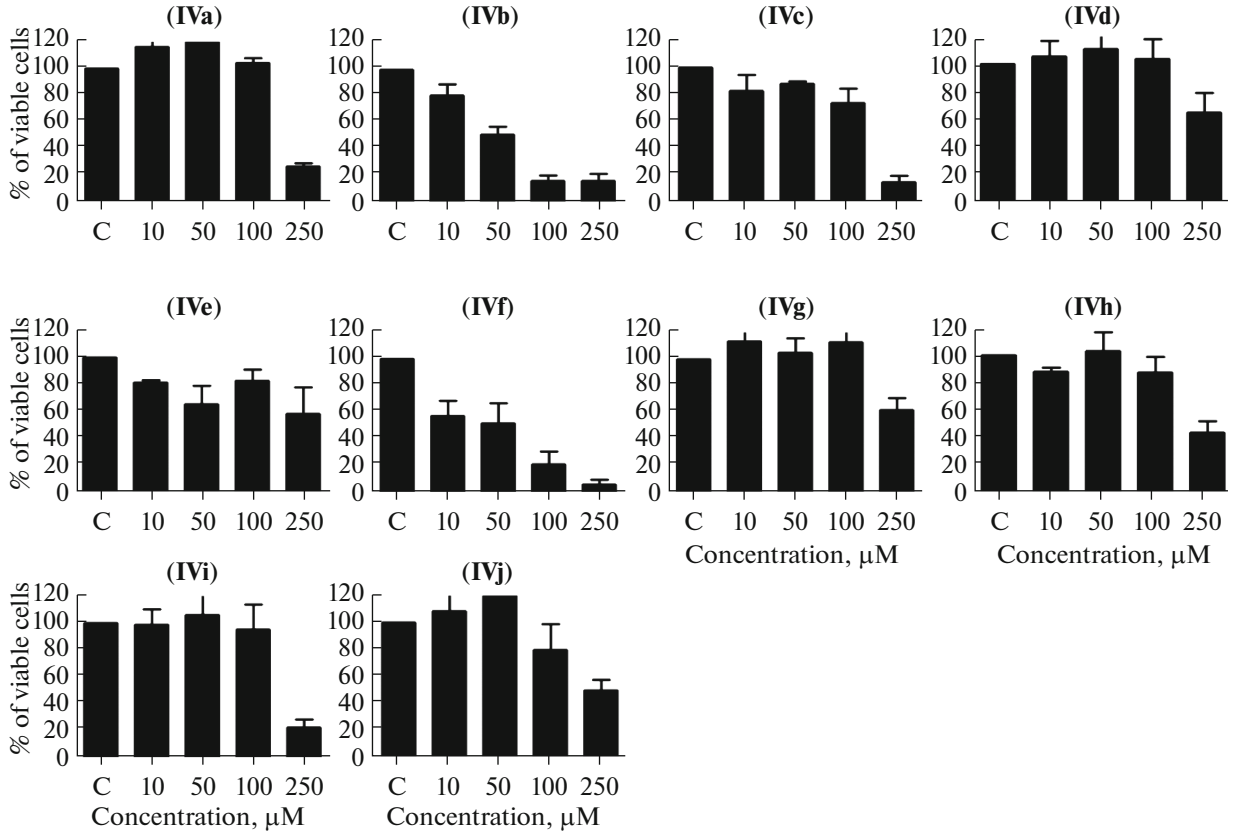


Fig. 2. Evaluation of cytotoxicity of (**IVa–j**) in T cell leukemic cell line, CEM. Bar diagram depicting cytotoxicity of (**IVa–j**) in CEM cells following treatment with increasing concentrations of the compounds (10, 50, 100 and 250 μM) for 48 h. DMSO treated cells was used as vehicle control. Bar graphs represent % of viable cells following treatment with compounds. Experiments were performed at least three times and plotted using GraphPad Prism software. Bar graphs represent mean ± SEM.

^{13}C NMR (DMSO-d_6, 400 MHz): δ = 165.8, 163.3, 154.8, 151.4, 148.8, 143.7, 134.9, 134.9, 130.5, 130.0, 125.8, 124.9, 106.6, 47.2, 29.9, 27.9; HRMS (ESI): (*m/z*) $[M + H]^+$ calcd. for $C_{16}H_{13}ClN_6O_3$: 373.0738, found 373.0733.

7-((5-(2-Fluorophenyl)-1,3,4-oxadiazol-2-yl)methyl)-1,3-dimethyl-1H-purine-2,6(3H,7H)-dione (IVj). Off white solid; R_f: 0.4; Yield: 84%; Melting Point: 280–282°C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 8.08 (s, 1H), 7.88 (s, 1H), 7.81 (d, *J* = 7.6 Hz, 1H), 7.63 (d, *J* = 8 Hz, 1H), 7.52 (d, *J* = 7.6 Hz, 1H), 5.16 (s, 2H), 3.42 (s, 3H), 3.20 (s, 3H); ^{13}C NMR (DMSO-d_6, 400 MHz): δ = 166.1, 164.4, 154.8, 151.4, 148.4, 144.2, 134.6, 133.7, 132.1, 130.9, 127.7, 126.6, 106.8, 47.2, 29.4, 27.8; HRMS (ESI): (*m/z*) $[M + H]^+$ calcd. for $C_{16}H_{13}FN_6O_3$: 357.1067, found 357.1063.

BIOLOGICAL EVALUATION

Cell Culture

CEM (T cell leukemia) and Nalm6 (pre B cell leukemia) cells were cultured in RPMI 1640 containing 5% FBS and supplemented with 100 μg/mL of antibiotics Penicillin G and streptomycin. Similarly, HeLa (human cervical cancer) was cultured in DMEM containing 5% FBS and PenStrep. Cells were incubated in a humified atmosphere at 37°C containing 5% CO_2. HeLa and CEM were obtained from National Centre for Cell Science, Pune, India whereas Nalm6 was from M. Lieber, USA.

Cytotoxicity

Trypan Blue dye exclusion assay was used to evaluate effect of the compounds in cancer cell lines as described in previous studies [19, 20]. 25000 cells/mL were treated with increasing concentrations of the compounds (10, 50,100 and 250 μM). Control cells were treated with an equivalent amount of DMSO since the compounds were dissolved in the latter. After 48 h of incubation with the compounds, cytotoxicity was evaluated and IC_{50} values calculated for each compound in three different cell lines, HeLa, CEM and Nalm6. Equal volume of 0.4% Trypan blue (Sigma Chemical Co., St Louis, MO, USA) and cells were mixed and counted using haemocytometer as well as Luna-II automated cell counter. Experiments were

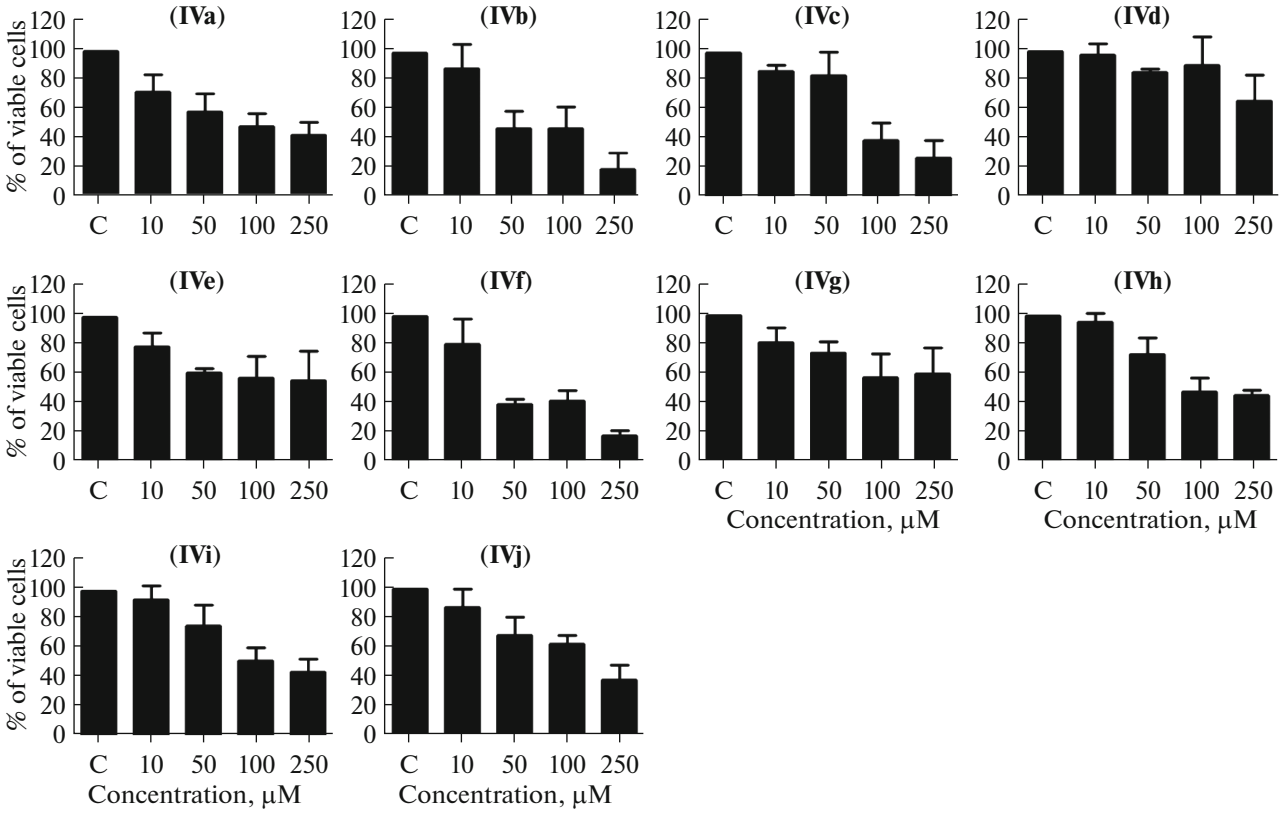


Fig. 3. Assessment of cytotoxicity of **(IVa–j)** in pre B leukemic cell lines after 48 h of treatment. Cytotoxicity induced by **(IVa–j)** in Nalm6 cells were evaluated following treatment (for 48 h) with increasing concentrations of compounds (10, 50, 100 and 250 μM). DMSO treated cells served as vehicle control. Bar graphs represent % of viable cells in each treatment conditions. Experiments were performed at least three times and plotted using GraphPad Prism software. Bar graphs represent mean ± SEM.

repeated at least three times and error bars were plotted using GraphPad Prism.

CONCLUSION

In conclusion, we have successfully synthesized 10 novel theophylline methyl 1,3,4-oxadiazole derivatives in moderate to excellent yields, all synthesized compounds were subjected to cytotoxic studies. Among the 10 oxadiazole derivatives, several compounds showed good cytotoxicity in three cancer cell lines. **(IVb)** and **(IVf)** showed 6.25–8.6 fold better cytotoxicity than SCR7 in T cell leukemic cell lines, CEM. When compared among the cell lines again **(IVb)** and **(IVf)** showed promising effect than other compounds of the series. Thus, these two derivatives have the potential for developing as anticancer agents.

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COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain any studies involving human participants performed by any of the authors and does not contain any studies involving animals performed by any of the authors.

Conflict of Interests

The authors declare that they have no conflicts of interest.

SUPPLEMENTARY MATERIALS

Supplementary materials are available for this article at https://doi.org/10.1134/S106816202005009X and are accessible for authorized users.

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