Available online at **www.joac.info**

ISSN: 2278-1862

Journal of Applicable Chemistry

2019, 8 (3): 1084-1090

(International Peer Reviewed Journal)

Synthesis, Characterization and anticancer study of 1-(1-(2-chloro-4-nitrophenyl)-5-methyl-*1H*-1,2,3-triazol-4-yl)ethanone

C. H. Aminath Rajeena* and Suresh P. Nayak

Department of Post-Graduate Studies and Research in Chemistry, Mangalore University, Mangalagangothri-574 199, Karnataka, **INDIA**

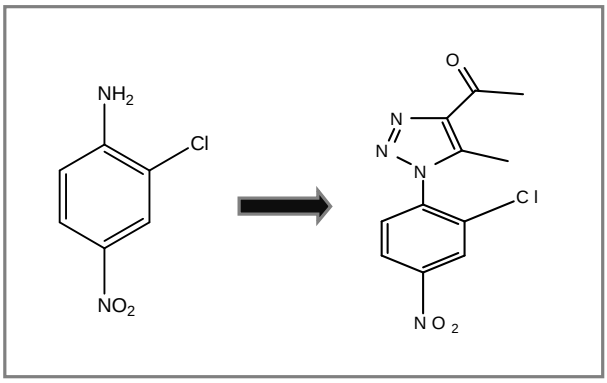
Email: rechunaa@gmail.com

Accepted on 8th April, 2019

ABSTRACT

In an effort to develop anticancer agents, in the present study 1,2,3 triazole linked ketone (1-(1-(2-chloro-4-nitrophenyl)-5-methyl-1H-1,2,3-triazol-4-yl)ethanone) was synthesized by taking 2-chloro-4-nitro aniline as a starting material. The above compound was synthesized in a good yield and characterized using FT-IR, LCMS, ^{1}H NMR and ^{13}C NMR spectral studies. The synthesized compound showed promising cytotoxicity on A549 lung adenocarcinoma cells, as evaluated using MTT assay.

Graphical Abstract

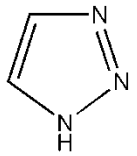


Keywords: 2-chloro-4-nitro aniline, 1,2,3 triazoles, Anticancer activity, MTT assay.

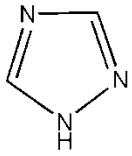
INTRODUCTION

Cancer is reported as one of the Global Burden of Diseases (World Health Organization 2008) and in the coming years as per the survey it will be one of main causes of death. Therefore, great attention needs to be given towards prevention and treatment of cancer with respect to the effect of disease for long term well-being of individuals. Scientists and physician are struggling hard to find better treatment for these kinds of diseases. Therefore, the search for novel and better anticancer agents has become one of the prime targets in drug discovery.

The chemistry of N-bridged heterocyclic compounds, such as triazole, has enhanced researchers to synthesize more and more heterocyclic compounds [**1**]. A triazole is a five-membered ring having two carbon atoms and three nitrogen atom with molecular formula $C_2H_3N_3$.They exist in two isomeric forms 1,2,3 triazole and 1,2,4 triazole which differ in their relative positions of nitrogen atoms[**2**].



1,2,3 -Triazole



1,2,4-Triazole

Among these derivatives, little work has been done in the areas of 1,2,3 triazoles and these compounds are very much effective in hydrogen bond formation which increases their solubility and capability to interact with biomolecular targets. Since 1,2,3-triazoles has three adjacent nitrogen atoms, compared to other compounds these are highly immutable to metabolic degradation [**3**] and these compounds are being appreciated for their industrial applications like corrosion inhibitors [**4**], sensors and photostabilizers [**5**]and also incorporated in wide range of therapeutically interesting drugs like H1/H2 histamine receptor blockers [**6**], CNS stimulants, anti-anxiety agents [**7**], sedatives as well as selective β_3-adrenergic receptor agonists [**8**].

Triazole is considered to be an effective scaffold possessing diverse pharmacological properties like antifungal [**9**], antibacterial [**10**], anti-HIV [**11**], anticonvulsants [**12**] and anti-allergic [**13**], antitubercular [**14**], anticancer [**15**], anti-inflammatory [**16**] etc. Keeping this in mind, in the present work we focused on the synthesis of 1-(1-(2-chloro-4-nitrophenyl)-5-methyl-*1H*-1,2,3-triazole bearing ketone and found to have cytotoxic effect against cancer cell lines.

MATERIALS AND METHODS

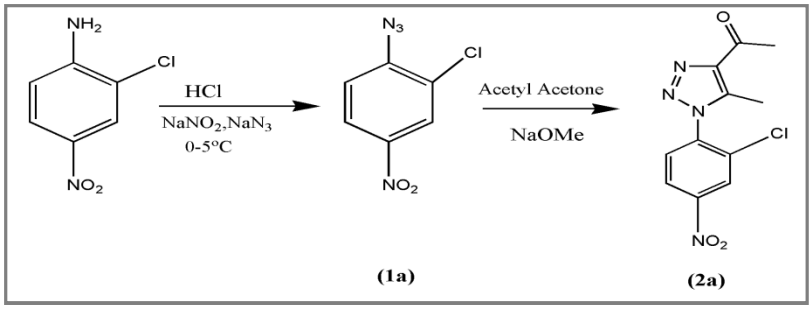
The starting material, 2-Chloro-4 nitro aniline was purchased from sigma Aldrich and used after purification. All the other chemicals and solvents were purchased from either Sigma Aldrich or Merck. Reagents used here are of analytical grade. IR spectrum was determined using SHIMADZU FT-IR 157 spectrophotometer and frequencies were expressed in cm^{-1}.^{1}H NMR and ^{13}C NMR spectra were procured using Bruker Advance II NMR spectrometer operating at 400MHz using $CDCl_3$ as solvent. The chemical shift (*J*) values were expressed in parts per million (ppm) in relative to internal standard TMS (δ=0). Multiplicity is symbolized as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m). Mass spectrum was acquired on a SHIMADZU LCMS-8030 mass spectrometer. Melting points were determined in open capillary tubes using Innovative DTC-967A digital melting point apparatus are uncorrected. Completion of reactions was monitored and checked by TLC.

Synthesis of 1-azido-2-chloro-4-nitrobenzene (1a): 2-chloro-4-nitro aniline (5 g) was heated to dissolve in a mixture of HCl and water (1:2), and then cooled in an ice bath (0-5°C). To this cold solution, $NaNO_2$ (0.03 mol) and NaN_3 (0.03 mol) were added drop wise. The above solution was kept aside for a day for the completion of diazotization. Further the solution was extracted with chloroform (30 mL), then with salt water (a spatula of salt in 100 mL water). The organic layer thus separated was then treated with a pinch of sodium sulphate (anhydrous) to remove water traces and the azide was kept aside for evaporation of chloroform. Yield (70%)

Synthesis of 1-(1-(2-chloro-4-nitrophenyl)-5-methyl-1-*H*-1,2,3-triazol-4-yl)ethanone (2a): Azide (2 g) was dissolved in methanol (10 mL), and kept under stirring. To this, methanolic sodium methoxide (0.012 mol in 10 mL methanol) and acetyl acetone (0.01 mol) was added and continued stirring for overnight. The resultant solution was poured into ice cold water, and then the precipitate

thus formed was filtered, dried and recrystallized from ethanol. Yield: 62%, M.P: 83-86°C, Color of the compound: Dark Pink

Synthetic Route:



Scheme 1. Synthesis of 1(1-(2chloro-4-nitrophenyl)-5-methyl-1H-1,2,3, trizol-4-yl)ehanone (2a).

RESULTS AND DISCUSSION

The synthetic pathway for the preparation of the title compound is illustrated in scheme 1. The title compound 1-(1-(2-chloro-4-nitrophenyl)-5-methyl-*1H*-1,2,3-triazol-4-yl)ethanone (2a) was prepared via 1,3 cyclo addition of aryl azide with acetyl acetone in a good yield by taking 2-chloro 4-nitro aniline as a starting material. The structure of the compound was ascertained by IR, LCMS, ^{1}H NMR and ^{13}C NMR spectroscopy.

The FT-IR Spectrum of the title compound showed sharp band at 3100 cm^{-1} which corresponds to aromatic C-H stretching frequencies (Figure 1). The absorption band which appears at 1684 cm^{-1} was due to α-β unsaturated carbonyl system. Absorption band of C-NO_2 appeared at 1352 cm^{-1} and C-Cl band at 740 cm^{-1}. The 400MHz ^{1}H NMR spectrum of 2a showed a singlet at δ 2.50 and δ 2.71 integrating for three protons of CH_3 group which corresponds to protons of $COCH_3$ and triazole ring (Figure 2). ^{13}C NMR spectrum of the compound showed δ 9.61, δ 27.89, which corresponds to carbon atoms of CH_3 of triazole ring and $COCH_3$, δ 193.97 corresponds to C=O carbon (Figure 3). The mass spectrum of compound 2a showed isotopic peak at m/z 281 (M^+) and 283 (M^++2) in the ratio 3:1 in consistent with their molecular formula $C_{11}H_9ClN_4O_3$ (Figure 4).

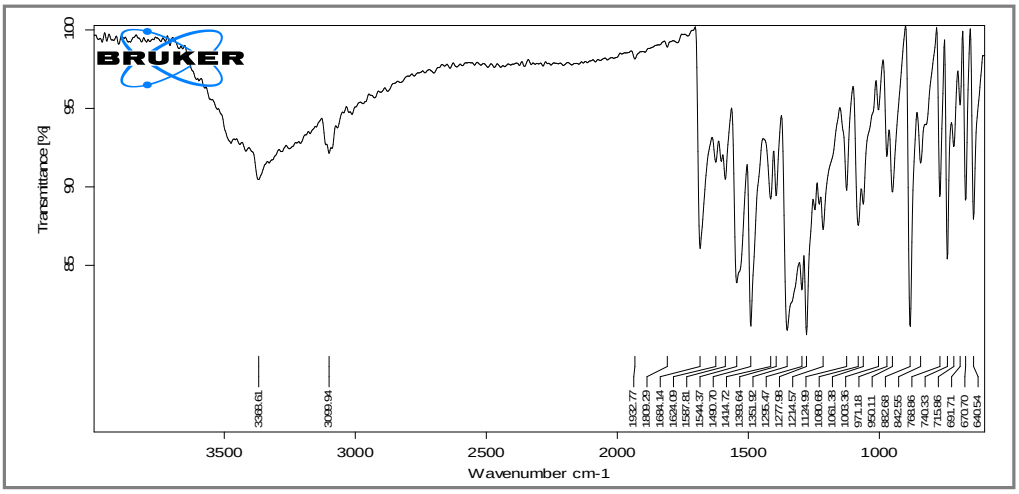


Figure 1. FT-IR Spectrum of Compound 2a.

IR (KBr,cm^{-1}) : 3100 (Ar C-H), 1684 (C=O), 1352(C-NO_2), 740 (C-Cl) ; **^{1}HNMR** ($CDCl_3$,400 Hz): δ 2.50 (s,3H, $COCH_3$), δ 2.71 (s,3H, triazole ring CH_3), δ 7.67 (d, 1H, *J* = 8.64Hz, Ar-H), δ 8.39 (dd,1H, *J* = 2.44 Hz and *J* = 8.68Hz, Ar-H), δ 8.53 (d,1H, *J* = 2.40 Hz, Ar-H) ; **^{13}C NMR** ($CDCl_3$,100 MHz): 9.61, 27.89, 123.10, 126.06, 130.24, 133.31, 138.09, 139.19, 143.38, 149.34, 193.97 ; **LC-MS**: m/z: 281(M^+), 283 (M^++2); Molecular Formula: $C_{11}H_9ClN_4O_3$, Calculated Formula Weight: 280.66 (Figures 1-4)

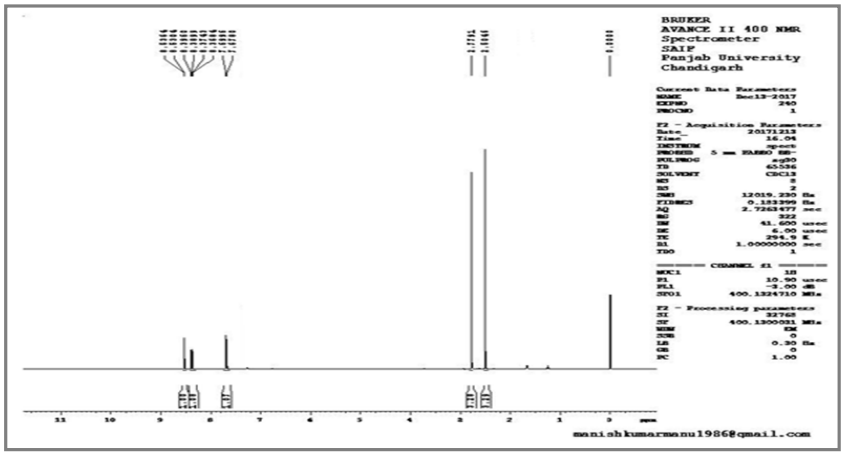


Figure 2. ^{1}H NMR Spectrum of Compound 2a.

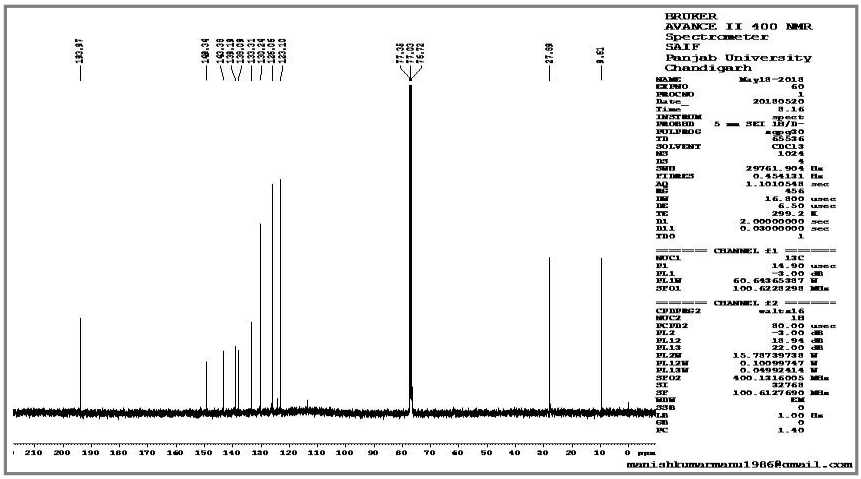


Figure 3. ^{13}C NMR Spectrum of Compound 2a

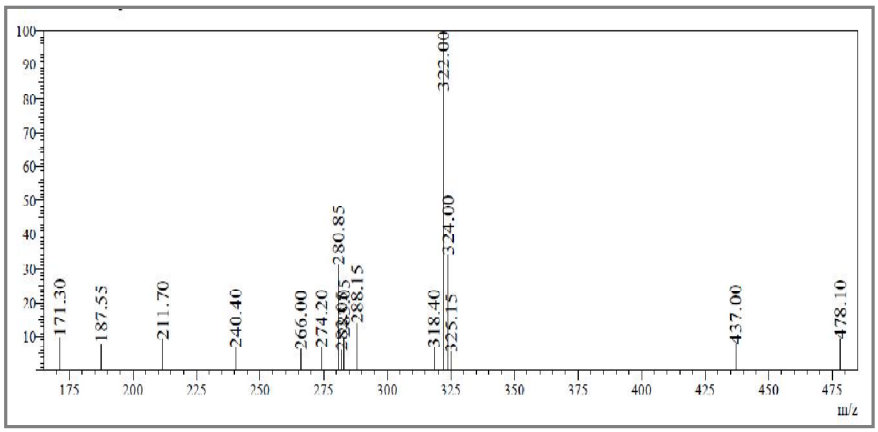


Figure 4. Mass spectrum of the compound 2a.

APPLICATION

The main purpose of the present work is to synthesize a novel nitrogen heterocycles which exhibits good anticancer activity. So in the present work, the synthesized triazole ketone was evaluated for their anticancer studies.

Anticancer Studies (in-vitro): Cytotoxicity of the 1-(1-(2-chloro-4-nitrophenyl)-5-methyl-1-*H*-1,2,3-triazol-4-yl)ethanone on lung adenocarcinoma cells was assessed using MTT assay.

Cells and Culture conditions: Human lung adenocarcinoma cells (A549) were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10 % fetal bovine serum and 1 % antibiotic-antimycotic solution. They were incubated at 37ºC under 5% CO_2, subcultured upon attaining 70 % confluence and used for the experiments after three consecutive passages.

Cytotoxicity assessment using Methyl Thiazolyl Tetrazolium (MTT) assay: Cytotoxicity of the compounds on A549 cells was evaluated using MTT assay (Mosmann, 1983). Cells were seeded at a density of 5000 cells/well in 96 well microtiter plates and incubated at 37°C under 5% CO_2 in a humidified atmosphere for 24 h. Test compounds were added to the cells at concentrations of 6.125, 12.5, 25, 50 and 100 µg mL^{-1} and incubated further for 48 h. 100 µL of MTT solution (1mg mL^{-1}) was added to the wells and incubated for 4h. Formazan crystals were solubilized in DMSO and absorbance was recorded at 570 nm using multimode microplate reader (Fluo STAR Omega, BMG Labtech).

Results of anticancer studies (Table 1) indicated that the compound possessed significant anticancer activity on lung cancer cells, with an IC_{50} value of 42.76 µg mL^{-1} (Table 1). Activity was found to be concentration dependent, with the highest cytotoxicity at 100 µg mL^{-1} concentration being 85.3%. There are studies reporting the anticancer activity of triazole derivatives. 1,2,3 triazoles showed anticancer efficacy on melanoma, colon and breast cancer cell lines [**17**]. Mono 1,2,3-triazole analogs exerted significant anticancer activity on murine melanoma cells [**18**]. 1,2,4-triazolo[3,4-b]-1,3,4-thiadiazine derivatives showed cytotoxicity on liver, breast and uterine cervix cancer cells with IC_{50} values of 6.50, 8.59 and 6.00 µM respectively [**19**]. However, there are no reports on the anticancer activity of 1-(1-(2-chloro-4-nitrophenyl)-5-methyl-*1H*-1,2,3-triazole. Thus, the results of the study indicated that the tested triazole derivative can be a potential anticancer agent after appropriate pre-clinical validations.

Table 1. Cytotoxicity of triazole derivative on A549 cells

Concentration (µg mL^{-1})	Cytotoxicity (mean% ± SD)	IC_{50} (µg mL^{-1})
6.125	10.8 ± 0.21	
12.5	23.3 ±0.21	
25	45.5 ± 0.32	42.76
50	69.4 ± 0.64	
100	85.3 ± 0.32	

CONCLUSION

Encouraged by the previous results of anticancer agents of 1,2,3 triazoles, an effort has been made to develop an anticancer agents with favorable activity. Based on the in-vitro anticancer studies, it has been observed that the compound 1-(1-(2-chloro-4-nitrophenyl)-5-methyl-1-*H*-1,2,3-triazol-4-yl)ethanone showed promising cytotoxicity, so this compound can be applied successfully to design better anticancer agents.

ACKNOWLEDGEMENTS

The authors are thankful to Director SAIF- Panjab University (Chandigarh) and to Head-DST PURSE and USIC (Mangalore University) for providing spectral data and YRC (Yenepoya Research Centre) for carrying out anticancer activity.

REFERENCES

[1]. V. S. Wakale, S. R. Pattan, V. Tambe, Therapeutic Importance of 1, 2, 4-Triazole: A Review, *Int. J. Res. Pharm. Biomed. Sci*., **2013**, 4 (3), 985-1001

[2]. P. Surendranath, P. Ashish, Rupesh, Triazole: a potential bioactive agent (Synthesis and biologiacal activity), *Int. j. Ayurveda pharma res.*, **2011**, 2(5), 1490

[3]. N. S. Vatmurge, B. G. Hazra, V. S. Pore,F. Shirazi, P. S. Chavanb, M. V. Deshpande, Synthesis and antimicrobial activity of b-lactam–bile acid conjugates linked via triazole, *Bioorganic Med. Chem. Lett.*, **2008**, 18, 2043-2047

[4]. T. Zhang, S. Cao, H. Quan, Z. Huang, S. Xu, Synthesis and corrosion inhibition performance of alkyl triazole derivatives, *Res Chem Intermed*., **2015**, 41, 2709-2724.

[5]. T. Wang, X. C. Hu, X. J. Huang, X. S. Li, J. W. Xie, Efficient Synthesis of Functionalized 1,2,3-Triazoles by Catalyst-Free 1,3-Dipolar Cycloaddition of Nitroalkenes with Sodium Azide, *J. Braz. Chem. Soc.*, **2012**, 23(6), 1119-1123

[6]. M. Wijtmans, C. de Graaf, G. de Kloe, E.P. Istyastono, J. Smit,H. Lim, R. Boonnak, S. Nijmeijer, R. A. Smits, A. Jongejan, O. Zuiderveld, I. J. P. Esch, R. Leurs, Triazole Ligands Reveal Distinct Molecular Features That Induce Histamine H_4 Receptor Affinity and Subtly Govern H_4/H_3 Subtype Selectivity, *J. Med. Chem.*, **2011**, 54 (6), 1693-1703

[7]. R. Kumar, M. Shahar Yar, S. Chaturvedi, Srivastava, A Triazole as Pharmaceuticals Potentials, *Int. J. PharmTech Res*, **2013**, 5(4), 1844-1869

[8]. L. L. Brockunier, E. R. Parmee, H. O. Ok, M. R. Candelore, M. A. Cascieri, L. F. Colwell, L. Deng, W. P. Feeney, M. J. Forrest, G. J. Hom, D. E. MacIntyre, L. Tota, M. J. Wyvratt, M. H. Fisher, A. E. Weber, Human beta3-adrenergic receptor agonists containing 1,2,3-triazole-substituted benzenesulfonamides, *Bioorg. Med. Chem. Lett*,.**2000**, 10, 2111-2114.

[9]. S. Senthil, R. Gopi,N-Substituted-1,2,3-triazoles: Synthesis, characterization and antimicrobial activity studies, *Der Pharma Chemica*, **2015**, 7(6), 15-23

[10]. F. Reck, F.Zhou, M. Girardot, G. Kern, C. J. Eyermann, N. J. Hales, R. R. Ramsay, M. B. Gravestock, Identification of 4-substituted 1,2,3-triazoles as novel oxazolidinone antibacterial agents with reduced activity against monoamine oxidase A, *J. Med. Chem.*,**2005**,48, 499-506

[11]. R. Alvarez, S.Velazquez, A.San-Felix, S. Aquaro, E. De Clercq, C. F. Perno, A.Karlsson, J. Balzarini, M. J. Camarasa, 1,2,3-Triazole-[2,5-Bis-O-(tert-butyldimethylsilyl)-.beta.-D-ribofuranosyl]-3'-spiro-5"-(4"-amino-1",2"-oxathiole 2",2"-dioxide) (TSAO) Analogs: Synthesis and Anti-HIV-1 Activity,*J. Med. Chem*,. **1994,** 37, 4185-4194.

[12]. P. K. Kadaba, Triazolines. 14. 1,2,3-Triazolines and triazoles, a new class of anticonvulsants. Drug design and structure-activity relationships, *J. Med. Chem.*, **1988**, 31(1), 196-203

[13]. D. R. Buckle, C. J. Rockell. H. Smith, B. A. Spicer, Studies on 1,2,3-triazoles. 10. Synthesis and antiallergic properties of 9-oxo-1H,9H-benzothiopyrano[2,3-d]-1,2,3-triazoles and their S-oxides, *J. Med. Chem.*, **1984**, 27(2), 223-227

[14]. J. M. Altimari, S. C. Hockey, H. I. Boshoff, A. Sajid, L. C. Henderson, Novel 1,4-substituted-1,2,3-triazoles as antitubercular agents, *Chem. Med. Chem.*, **2015**, 10(5), 787-791.

[15]. K. Marciniec,Latocha, R. Kurczab,S.Boryczka, Synthesis and anticancer activity evaluation of a quinoline-based 1,2,3-triazoles, *Med Chem Res.*, **2017**, 26, 2432-2442

[16]. R. J. Singh, 1, 2, 3-triazole derivatives as possible antiinflammatory agents, *Rasayan J. Chem.*, **2009**, 2, 706-708

[17]. N. Pokhodylo, O. Shyyka, V. Matiychuk,Synthesis of 1,2,3-Triazole Derivatives and Evaluation of their Anticancer Activity, *Sci Pharm*., **2013**, 81(3), 663-676

[18]. H. Elamari, R. Slimi, G. G. Chabot, L. Quentin, D. Scherman, C. Giard, Synthesis and in vitro evaluation of potential anticancer activity of mono and bis-1,2,3-triazole derivatives of bis-alkynes, *Eur J Med Chem.*, **2013**, 60, 360-364

[19]. A. Ahmad, H. Varshney, A. Rauf, A. Sherwani, M. Owais, Synthesis and anticancer activity of long chain substituted 1,3,4-oxadiazol-2-thione, 1,2,4-triazol-3-thione and 1,2,4-triazolo[3,4-b]-1,3,4-thiadiazine derivatives, *Arab J Chem.*, **2017**, 10, 3347-3357.