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In-vitro cytotoxic evaluation, hemolytic and thrombolytic potential of newly designed acefylline based hydrazones as potent anticancer agents against human lung cancer cell line (A549)

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Abstract: Hydrazones of theophylline-7-acetic acid 5a-g have been synthesized using ultrasonic irradiation as well as conventional heating system and analyzed for their anticancer characteristics against human lung cancer cell line (A549). Compound 5g with cell viability 33.19±0.49% (100 µg/µL) compared to the starting reference drug acefylline having cell viability 86.32±11.75% (100 µg/µL) was found to be the most active anticancer agent among all. The synthesized derivatives were also exposed to hemolytic and thrombolytic studies to determine their cytotoxic profile and results revealed their low toxicity and moderate clot lysis activity.

Keywords: Hydrazones, acefylline, cell viability, hemolytic, thrombolytic.

INTRODUCTION

Methylxanthines, such as theophylline, caffeine and theobromine, are derived from plants and are well acknowledged for its biological and pharmacological significance (Monteiro *et al*., 2016). Theophylline has been considered potent bronchodilator for decades and has also been used as respiratory stimulator for the treatment of acute pulmonary edema (Ruddarraju *et al*., 2019). Moreover, due to its appealing characteristics, availability and safety, scientists are constantly working to explore theophylline and its derivatives in medicine and pharmaceuticals (Matera *et al*., 2017). Acefylline, being derivative of theophylline, was prepared to evade the side effects of theophylline drug and fortunately it was found less toxic and also that kidneys excrete acidic compounds which can enhance clearance rate (Stavrakov *et al*., 2016). Ester derivatives of acefylline showed comparable broncholytic activity and three times lower toxicity than standard aminophylline (Rogliani *et al*., 2019). Another acefylline derivative, 7-theophyllineacetyloxyglycol, shows higher broncholytic potential than standard and has no cardiac side effect (Singh *et al*., 2018). Furthermore, by introducing new moieties at acefylline scaffold such as peptide linkage can significantly enhance cytotoxic effect of acefylline (Rogliani *et al*., 2019).

Similarly, the biological potential of Schiff bases is also well recognized. This is attributed to the toxophoric C-N bond between them (Mishra *et al*., 2005). Schiff bases containing azomethine (-CH=N) moiety are the compounds which demonstrate a strong classification of biological activities such as anti-inflammatory, antibacterial and anticancer activities. The discovery of anti-proliferative drugs has resulted in the discovery of several hydrazones with antitumor potential (Rollas and Kucukguzel, 2007). Our research group has previously reported the biological potential of acefylline based 1,3,4-oxadiazoles (Shahzadi *et al*., 2020) and 1,2,4-triazoles (Shahzadi *et al*., 2021) as anti-cancer agents. In view of the above facts and as part of our ongoing research, we found it interesting to explore the cytotoxic potential of acefylline based hydrazones. For this purpose, hydrazide derivative of acefylline was allowed to react with various aromatic aldehydes and resulting hydrazones were tested for their cytotoxity against A549 lung cancer cell line. The hemolytic and thrombolytic potential of the synthesized derivatives was also studied.

MATERIALS AND METHODS

IR spectra (ν, cm−1) of the synthesized compounds were recorded on Bruker FTIR spectrometer (in KBr pellets). NMR spectra were obtained on a Bruker Spectrometer model *AV*-400 at 400 Mega Hertz. Melting points of the synthesized compounds were found in glass capillaries by using Gallenkamp equipment. The synthesized compounds were purified by column chromatography and recrystallization method using ethanol and dichloromethane as solvents. TLC performed on pre-coated silica gel 60 F254 plates using analytical grade solvents like methanol, dichloromethane.

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General method for the synthesis of acefylline derived hydrazones 5a-g

Method 1: Conventional method

An equimolar mixture of theophylline acetohydrazide and the corresponding aldehydes were heated under reflux with few drops of acetic acid in ethanol (50 ml) for 2 hours. After completion of reaction, the reaction mixture was cooled and the solid obtained was filtered, which was dried and recrystallized using ethanol to give corresponding hydrazones of acefylline.

Method 2: Ultrasonic assisted method

An equimolar mixture of theophylline acetohydrazide and the corresponding aldehydes were heated into a sonicator at 50°C for 30-40 min with few drops of acetic acid in ethanol (50 ml). The solid obtained was filtered which was dried and recrystallized using ethanol to obtain corresponding hydrazones of acefylline.

MTT assay

The cytotoxity of the target compounds was tested by MTT assay according to reported procedure (Akhtar *et al*., 2020; Akhtar *et al*., 2021). The prepared derivatives were scrutinized against human lung cancer cell lines. The A549 cell line was incubated as a monolayer culture in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% lethal bovine serum and Penicillin/ Streptomycin1% (100 µg/mL). It was then subjected to 95% humid air with 5% CO_2 at 37°C.Cells were treated with the appropriate concentration of dissolved compounds in DMSO. In all experiments, cells treated with dimethyl sulfoxide were used as controls. A549 (lung) cell lines were grown overnight in 96-well plates and were treated with different concentrations of compounds for 48 hours, MTT reagent was added (10 µl, 5 mg/mL) and cells were further incubated for 4 hours at 37°C. Then, 150 µl DMSO was added in each plate and farmazan crystals were dissolved, the absorbance was measured in a Thermo Scientific microplate reader at 490 nm to calculate the percentage of cell viability (Hafeez *et al*., 2021).

Hemolytic assay

A 3 mL sample was taken from human blood, centrifuged for 5 minutes at 1000 ×g. Erythrocytes were isolated dissolved in phosphate buffer (pH 7.4) salts and washed. Synthetic compounds solution (10 mg/mL) was added (20 µL) to 180 µL of RBC suspension and refrigerated for 30 minutes. ABTS was taken as a positive control and DMSO as negative control. % age hemolysis was calculated by using the formula (Riaz *et al*., 2012).

$$\%\,\text{age of hemolysis} = \frac{\text{Absorbance of sample - Absorbance of negative control (DMSO)}}{\text{Absorbance of positive control (ABTS)}} \times 100$$

Thrombolytic assay

1 mL blood sample of human was incubated to pre-weighed tubes at room temperature for 45 minutes. After clotting, the serum was totally removed and all eppendorfs were reweighed to determine the clot weight. Sample (100 mL) was added and placed at 37°C for 3 hours and clot lysis was observed. DMSO was also added in a patch consisting of tubes that acted as a negative thrombolytic control while ABTS as positive control. Eppendorfs were weighed again and with a difference in weight after clotting percentage of thrombolysis was calculated (Batool *et al*., 2018).

$$\text{Percentage of clot lysis (\%)} = \frac{\text{Initial clot weight - Final clot weight}}{\text{Initial weight of clot}} \times 100$$

STATISTICAL ANALYSIS

The experiments were executed in triplicate. The data was evaluated using the statistical software SPSS version 23.0. The results of averages and dispersion in the data were compared using statistical test ANOVA and presented in terms of Mean ± SD in table 2.

RESULTS

Acetohydrazide hydrazones of theophylline were synthesized and presented in scheme 1. By condensation of acefylline 1 with methanol using sulphuric acid as catalyst, theophylline-7-acetate 2 was obtained in 70%, hydrazine monohydrate was added to get theophylline-7-acetohydrazide 3 in 99% yield according to reported procedure (Shahzadi *et al*., 2020). The synthesized hydrazide 3 was then treated with corresponding aromatic aldehydes 4a-g in ethanol and acetic acid (few drops) in sonicator, the target acetohydrazide hydrazones of theophylline 5a-g (Scheme 1, table 1) were synthesized in 70-80 % yield (Alrasheed *et al.,* 2016).

DISCUSSION

The synthesis of target compounds hydrazones of acefylline 5a-g was achieved in 60-70% yield by applying conventional heating method under reflux for 02 hours while same reactions were carried out in sonicator for 30-40 minutes and desired derivatives were achieved in 70-80% yield within short time. All the synthesized compounds were characterized by IR and NMR.

Spectral analysis of molecule (5f)

Compound 5f was synthesized as cream solid, structure was confirmed by ^{1}H-, ^{13}C NMR, IR and by molecular ion peak (M^+) at m/z 434.0341in MS-EI spectrum. Various absorption spectra were used to describe different functional groups in FT-IR at υ3354 (N–H); 1644 (CO-amide); 1650 (CO-xanthene); 1546 (C=N); Ph (1476); 1330 (C–N); 1453 (C=C) cm^{-1}. The most downfield signal in ^{1}H-NMR spectrum was detected at δ 11.06 as singlet for 1H, of CONH and at δ 8.69 for azomethine (N=CH)N-H of the heterocyclic xanthene ring.

Table 1: Spectral data of compounds 5a-g

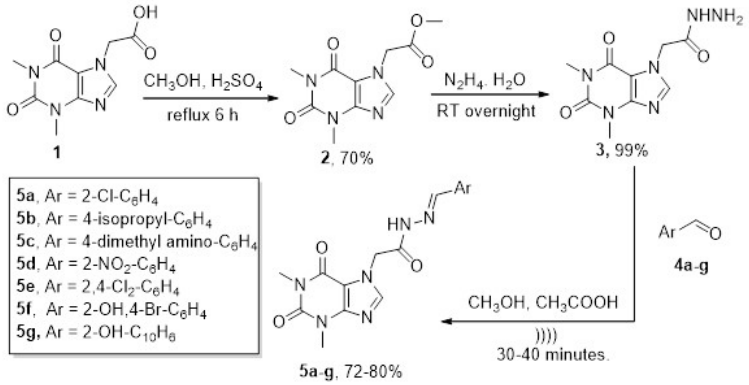
Compound	M.P. (°C)	Yield (%)	FT-IR (KBr, cm^{-1})ν_{max} / ^{1}H-NMR (DMSO-d_6, 400 MHz)/ MS (*m/z*)
5a	295	73	1600-1650 (CO–xanthene), 3351 (N-H), 1643 (CO–amide), 1544 (C=N), 1453 (C=C), 1330 (C–N), Ph (1476)/ 3.17, 3.43 (s, 6H, NCH_3), 5.75 (s, 2H, NCH_2), 7.39-7.76 (m, 4H, Ar-H), 7.96, 8.78 (s, 2H, N=CH), 11.02 (s, 1H, CONH)/374.0881[M^+]
5b	230	77	1600-1650 (CO–xanthene), 3354 (N-H), 1644 (CO–amide), 1546 (C=N), 1453 (C=C), 1331 (N– C), Ph (1476)/ 1.20 (s, 6H, CCH_3), 2.78 (s,1H, $CHCH_3$), 3.32, 3.40 (s, 6H, NCH_3), 5.24 (s, 2H, NCH_2), 7.22 (d, $J_{2',3'} = J_{6',5'}$ 6Hz, 2H, H-5' and H-3'), 7.60 (d, $J_{3',2'} = J_{5',6'}$ 8Hz, 2H, H-6' andH-2'), 8.46, 8.54 (s, 2H, N=CH), 11.06 (s, 1H, CONH)/382.1749[M^+]
5c	260	79	1600-1650 (CO–xanthene), 3354 (N-H), 1644 (CO–amide), 1546 (C=N), 1453 (C=C), 1331 (N– C), Ph (1476)/ 3.08 (s, 6H, N-CH_3), 3.32, 3.39 (s, 6H, N-CH_3), 5.27 (s, 2H, NCH_2), 7.22 (d, $J_{2',3'} = J_{6',5'}$ 6Hz, 2H, H-5 and H-3'), 7.60 (d, $J_{3',2'} = J_{5',6'}$ 8Hz, 2H, H-2' and H-6'), 8.49, 8.74 (s, 2H, N=CH), 11.07 (s, 1H, CONH)/383.1711[M^+]
5d	240	75	1600-1650 (CO–xanthene), 3354 (N-H), 1644 (CO–amide), 1546 (C=N), 1453 (C=C), 1331 (N– C), Ph (1476)/ 3.15, 3.40 (s, 6H, NCH_3), 5.75 (s, 2H, NCH_2), 7.3-8.4 (m, 4H, Ar-H), 8.36, 8.56 (s, 2H, N=CH), 11.12 (s, 1H, CONH)/385.1128[M^+]
5e	310	72	1600-1650 (CO–xanthene), 3354 (N-H), 1644 (CO–amide), 1546 (C=N), 1453 (C=C), 1331 (N– C), Ph (1476)/ 3.32, 3.40 (s, 6H, NCH_3), 5.24 (s, 2H, NCH_2), 6.96 (d, $J_{5',6'}$ = 6 Hz, 1H, H-5'), 6.98 (s, 1H, H-3'), 7.21 (d, $J_{5',6'}$ = 6.4 Hz, 1H, H-6'), 8.42, 8.69 (s, 2H, N=CH), 11.06 (s, 1H, CONH) /408.0510[M^+]
5f	242	80	1600-1650 (CO–xanthene), 3354 (N-H), 1644 (CO–amide), 1546 (C=N), 1453 (C=C), 1331 (N– C), Ph (1476)/3.32, 3.40 (s, 6H, NCH_3), 5.24 (s, 2H, NCH_2), 5.75 (s, 1H, OH), 7.14 (d, $J_{5',6'}$ = 6 Hz, 1H, H-5'), 7.51 (s, 1H, H-3'), 7.55 (d, $J_{6',5'}$ = 6.4 Hz, 1H, H-6'), 8.42, 8.69 (s, 2H, N=CH), 11.06 (s, 1H, CONH)/434.0341[M^+]
5g	280	79	600-1650 (CO–xanthene), 3354 (N-H), 1644 (CO–amide), 1546 (C=N), 1453 (C=C), 1330(N–C), Ph (1476)/ 3.30, 3.36 (s, 6H, NCH_3), 5.37 (s, 2H, NCH_2), 5.75 (s, 1H, OH), 7.27-8.07 (m, 6H, Ar-H), 8.42, 8.69 (s, 2H, N=CH), 11.05 (s, 1H, CONH), 14.23/406.1401[M^+]

Cytotoxic potential

Table 2: Anticancer, hemolytic and thrombolytic activity of compounds 5a-g

S. No	Compounds	*Cell viability A549 (lung cancer)	%Hemolysis (Mean ± SD)	%Thrombolysis (Mean ± SD)
1	5a	59.01 ± 4.68	15.35± 0.02	44.60± 0.04
2	5b	91.06 ± 1.14	0.75± 0.01	47.24± 0.01
3	5c	58.45 ± 3.57	8.1± 0.03	47.44± 0.02
4	5d	71.13 ± 5.02	9.67± 0.01	44.26± 0.03
5	5e	56.77 ± 6.56	17.15± 0.05	49.24± 0.05
6	5f	57.50 ± 0.30	11.76± 0.02	45.68± 0.02
7	5g	33.19 ± 0.49	25.03± 0.04	59.87± 0.03
8	Acefylline	86.32 ± 11.75	43.5± 0.02	6.85± 0.04
9	DMSO (-ve control)	100 ± 0	0.01± 0.01	0.57± 0.01
10	ABTS(+ve control)		95.9± 0.02	86± 0.01

*Cell viability: (Mean ± SD (standard deviation), using 100 µg/µl concentration in triplicate).



Scheme 1: Pathway for the synthesis of final compounds (5a-g).

In the up-field region 2H-4 of CH_2 group reverberated at δ 5.24 whereas 2 protons of purine ring (3H-1, 2) resonated at δ 3.32 and δ 3.40 as singlet. Hydroxyl group (OH) presence was confirmed at aromatic ring by a signal at δ 5.75. *H*-5' and *H*-6' of phenyl ring showed resonance at δ 7.14 and at δ 7.55 as a doublet (*J*=6 Hz and *J*=6.4 Hz respectively), while *H*-3' resonated as singlet at δ 7.51 fig. 1A.

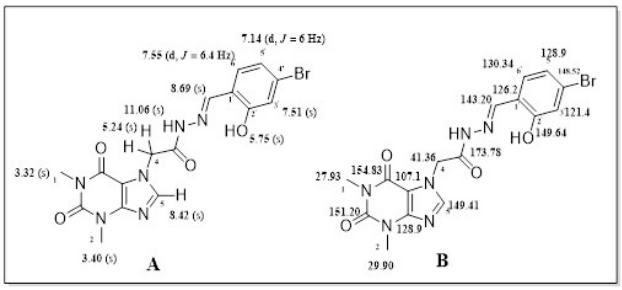


Fig. 1: A (^{1}HNMR) and B (^{13}C NMR) of compound (5f).

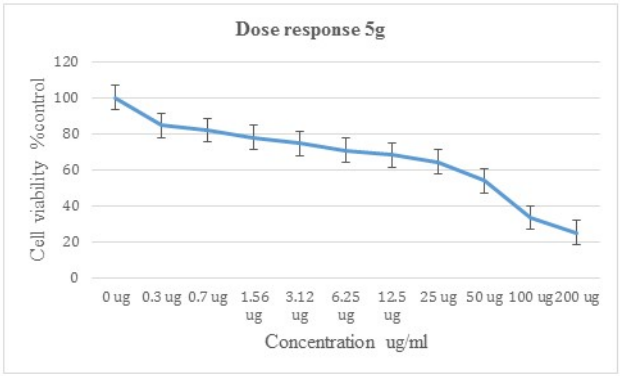


Fig. 2: Compound 5g made cell death in lung cancer cell line (A549) with indicated doses of 5g.

All the 16 carbons showed their signals in ^{13}C NMR spectrum, two resonance signals at δ 41.36and at δ 173.78 for methylene group and carbonyl group respectively confirmed the acetohydrazide of hydrazone. Two signals at δ 151.66 and 153.96 were belonged to purine ring (2C=O). A downfield signal portrayed by carbon at δ 143.20 confirmed the formation of Schiff base. Theophylline ring in the molecule was confirmed by one signal (methine) at δ 149.41 and the two signals (C=C) at δ 107.1 and 147.99, while the remaining six signals of substituted phenyl carbons appeared at δ 121.4-149.64 fig. 1B. From similar approach other synthesized compounds (5a-g) were also characterized.

Anticancer activity

The ability of synthetic derivatives to prevent the proliferation of A549 (lung) tumor cell lines was assessed by an MTT assay following literature methods (Shahzadi *et al*., 2021 and Irfan *et al*., 2022). To compare the cytotoxic activity of synthesized derivatives (5a-g), the starting drug acefylline was also studied in similar experimental situations. It was observed from the results that all the compounds except 5b (cell viability = 91.06± 1.14%) are more active against cancer cells than starting drug acefylline (cell viability = 86.32±11.75%). Compound 5g was found to be one of the potent anti-proliferative agent with cell viability (33.19±0.49%). All the remaining derivatives 5a, 5c, 5d, 5e and 5f (cell viability 59.01±4.68%, 58.45±3.57%, 71.13±5.02%, 56.77±6.56% and 57.50±0.30% respectively) were considered as moderately active agents against lung cancer cell line A549.

Compound 5g was applied at different concentrations (0.3-200 µg) and further screened for anti-cancer activity.

Structure activity relationship (SAR)

Compound 5g with 2-hydroxy substituted naphthalene ring showed greater potential (33.19±0.49%) against cancer cells. The activity was decreased to some extent when 2-hydroxy-4-bromo substituted phenyl ring was present in the molecule 5f (cell viability =57.50±0.30%), which shows that OH substituent containing phenyl rings are active against cancer but their activity increases with another phenyl ring as well as by the addition of electron withdrawing substituted phenyl ring at *para* position. Derivative 5e (cell viability = 56.77± 6.56%) having 2,4-dichlorophenyl (*ortho* and *para*) substituted ring was found to be second most active agent against cancer while the activity of compounds 5a having *ortho* chloro substituted phenyl ring and 5d having *ortho* nitro substituted phenyl ring with cell viability values (59.01±4.68% and 71.13±5.02% respectively) were slightly decreased when electron withdrawing substituted phenyl ring is only at *ortho* position. Compound 5c (cell viability =58.45±3.57%) having *N*, *N*-dimethylhylphenyl ring at para position is moderately active compound which shows that para position might be responsible for greater binding with cancer cell and is more active.

Hemolytic activity

All synthesized derivatives were exposed to hemolytic testing to determine their cytotoxic profile following prescribed method (Hafeez *et al*., 2021). The results of the percentage hemolysis shown in table 2 indicate that all derivatives are nearly non-toxic to the blood cell membrane compared to acefylline. Overall, very mild toxic molecules were found 5f (11.76±0.02%), 5d (9.67± 0.01%), 5c (8.1±0.03%), relative to DMSO and ABTS which contain 0.01±0.01% and 95.9±0.02% hemolysis, respectively. The maximum membrane toxic compound was observed to be 5g (25.03±0.04%) due to the substitution of the hydroxyl group at ortho position of naphthalene ring. Compounds 5e (17.15±0.05%) and 5a (15.35±0.02%) also show high toxicity due to presence of 2,4-dichlorophenyl ring and 2-chlorophenyl ring respectively while minimum was noted in compounds 5b (0.75±0.01%) in which the *p*-position of phenyl ring occupied by isopropyl group.

Thrombolytic activity

The results of the thrombolytic activity assessment revealed that all synthesized derivatives had moderate thrombolytic activity. Maximum clotlysis was observed in compound 5g (59.87±0.03%) as compared to acefylline itself (6.85±0.04%)while all other derivatives 5a, 5b, 5c, 5d, 5e and 5f (44.60±0.04%, 47.24±0.01%, 47.44± 0.02%, 44.26±0.03%, 49.24±0.05% and 45.68± 0.02%) exhibited low clotlysis activity compared to ABTS (86± 0.01%).

CONCLUSION

Facile synthesis of hydrazone derivatives of acefylline (5a-g) was carried out in good yield. All the compounds were subjected to find their cytotoxic potential against cancer cell line A549 (lung). All the compounds showed good activity against cancer cell line and compound 5g was found potent anticancer agent. Hemolytic and thrombolytic activity of the synthesized derivatives suggested their low toxicity and moderate clot lysis activity.

REFERENCES

Akhtar R, Zahoor AF, Rasul A, Ahmad M, Anjum MN, Ajmal M and Raza Z (2019). Design, synthesis, *In-silico* study and anti-cancer potential of novel *n*-4-piperazinyl-ciprofloxacin-aniline hybrids. *Pak. J. Pharm. Sci*., **32**(5): 2215-2222.

Akhtar R, Zahoor AF, Rasul A, Khan SG and Ali KG (2021). *In-vitro* cytotoxic evaluation of newly designed ciprofloxacin-oxadiazole hybrids against human liver tumor cell line (Huh7). *Pak. J. Pharm. Sci*., **34**(3): 1143-1148.

Al-Rasheed HH, Alshaikh MA, Khaled JM Alharbi NS and El-Faham A (2016). Ultrasonic irradiation: Synthesis, characterization, and preliminary antimicrobial activity of novel series of 4,6-disubstituted-1,3,5-triazine containing hydrazone derivatives. *J. Chem.*, pp.1-9.

Batool M, Tajammal A, Farhat F, Verpoort F, Khattak ZAK, Mehr-un-Nisa, Shahid M, Ahmad HA, Munawar MA, Zia-ur-Rehman M and Basra MAR (2018). Molecular docking, computational and antithrombotic studies of novel 1,3,4-oxadiazole derivatives. *Int. J. Mol. Sci*. **19**(11): 3606-3624.

Hafeez F, Mansha A, Zahoor AF, Ali KG, Khan SG and Naqvi SAR (2021). Facile green approach towards the synthesis of some phenyl piperazine based dithiocarbamates as potent hemolytic and thrombolytic agents. *Pak. J. Pharm. Sci*., **34**(5): 1885-1890.

Irfan A, Faiz S, Rasul A, Zafar R, Zahoor AF, Kotwica-Moizych K and Moizych M (2022). Exploring the synergistic anticancer potential of benzofuran-oxadiazoles and triazoles: Improved ultrasound- and microwave-assisted synthesis, molecular docking, hemolytic, thrombolytic and anticancer evaluation of furan-based molecules. *Molecules*, **27**(3): 1023-1045.

Matera MG, Page C and Cazzola M (2017). Doxofylline is not just another the ophylline. *Int. J. Chron. Obstruct. Pulmon. Dis*., **12**: 3487-3493.

Mishra P, Rajak H and Mehta A (2005). Synthesis of Schiff bases of 2-amino-5-aryl-1,3,4-oxadiazoles and their evaluation for antimicrobial activities. *J. Gen. Appl. Microbiol.*, **51**(2): 133-141.

Monteiro JP, Alves MG, Oliveira PF and Silva BM (2016). Structure-bioactivity relationships of methylxanthines: Trying to make sense of all the promises and the drawbacks. *Molecules*, **21**(8): 974-1006.

Rasul A, Millimouno FM, Malhi M, Tsuji I, Ali M, Li J and Li X (2013). Reactive oxygen species mediate isoalantolactone-induced apoptosis in human prostate cancer cells. *Molecules*, **18**(8): 9382-9396.

Riaz M, Rasool N, Bukhari IH, Shahid M, Zubair M, Rizwan K and Rashid U (2012).*In vitro* antimicrobial, antioxidant, cytotoxicity and GC-MS analysis of *Mazus goodenifolius. Molecules*, **17**(12): 14275-14287.

Rogliani P, Calzetta L, Ora J, Cazzola M and Matera MG (2019). Efficacy and safety profile of doxofylline compared to theophylline in asthma: A meta-analysis. *Multidiscip. Respir. Med*., **14**(25): 1-8.

Rollas S and Kucukguzel G (2007). Biological activities of hydrazone derivatives. *Molecules,* **12**(8): 1910-1939.

Ruddarraju RR, Kiran G, Murugulla AC, Maroju R, Prasad DK, Kumar BH, Bakshi V and Reddy NS (2019). Design, synthesis and biological evaluation of theophylline containing variant acetylene derivatives as α-amylase inhibitors. *Bioorg. Chem.*, **92**: 103120-103135.

Shahzadi I, Zahoor AF, Rasul A, Rasool N, Raza Z, Faisal S, Parveen B, Kamal S, Zia-ur-Rehman M and Zahid FM (2020). Synthesis, anti-cancer and computational studies of 1, 3, 4-oxadiazole-purine derivatives. *J. Heterocycl. Chem*., **57**(7): 2782-2794.

Shahzadi I, Zahoor AF, Rasul A, Mansha A, Ahmad S and Raza Z (2021). Synthesis, hemolytic studies, and *in silico* modeling of novel acefylline-1,2,4-triazole hybrids as potential anti-cancer agents against MCF-7 and A549. *ACS Omega*, **6**(18): 11943-11953.

Singh N, Shreshtha AK, Thakur MS and Patra S (2018).Xanthine scaffold: scope and potential in drug development in drug development. *Heliyon*, **4**: e00829.

Stavrakov G, Valcheva V, Voynikov Y, Philipova I, Atanasova M, Konstantinov S, Peikov P and Doytchinova I (2016). Design, synthesis and antimycobacterial activity of novel theophylline-7-acetic acid derivatives with amino acid moieties. *Chem. Biol. Drug. Des*., **87**(3): 335-341.