WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 2, 1148-1158. Research Article ISSN 2277– 7105

SYNTHESIS OF SOME NOVEL 1,2,3- TRIAZINE FUSED THIOPHENES AS PROMISING ANTITUBERCULAR AND ANTICANCER LEADS

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Article Received on
11 Dec. 2019,
Revised on 31 Dec. 2019,
Accepted on 21 Jan. 2020
DOI: 10.20959/wjpr20202-16744

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ABSTRACT

In the present investigation, the synthesis and antitubercular and anticancer screening of 1,2,3- triazine fused thiophenes are aimed at. In this study, a twelve new bicyclic/tricyclic thiene-1,2,3-triazenes-4-ones (ADVJS 2a-2l) were synthesized by diazotization reaction with 2-amino-3-N-substituted carboxamido-4,5- disubstituted thiophenes (ADVJS -1a – 1l). The structures of these newly synthesized compounds were characterized by IR and ^{1}H NMR studies. All the synthesized compounds were screened for their antitubercular and anticancer evaluation. From the present study it may be concluded that synthesized compounds are fruitful in terms of their structural novelty and marked biological activities.

KEYWORDS: Triazine, Thiophene, Antirubercular, Anticancer, diazotization reaction.

INTRODUCTION

Tuberculosis (TB) is a contagious disease caused by omnipresent mycobacteria i.e., *Mycobacterium tuberculosis.*[1] According to 2015 survey of WHO, the world had an estimated 10.4 million new TB cases. TB is one of the biggest killers striking people in their most productive years and accounts for 23% of the global TB burden in India alone.[2] The researchers have left no stone unturned to discover lead molecules against the disease even then no new chemical entity has appeared for use in clinical treatment of this disease over the last four decades.[3]

Cancer, the most debilitating disease, has advanced to such a level that it has become one of the universal cause of human suffering and death all over the world.[4,5] The huge arsenal of synthetic, semi-synthetic, and naturally-occurring agents for treating neoplastic diseases suffers from two major limitations; the first one being the lack of selectivity of conventional chemotherapeutic agents to cancer tissues, causing unwanted side effects.[6] The second is the acquisition of multiple-drug resistance by cancer cells to the available agents that impedes treatment of various kinds of cancer.[7] Therefore, developing novel molecules to circumvent multidrug resistances and exhibiting selective toxicity to cancer cells rather than to normal cells is need of the hour.

The triazine is a six-membered heterocyclic ring, analogous to the benzene ring but with three carbons replaced by nitrogens. 1,2,3-Triazines are a class of biologically active compounds that exhibit a broad spectrum of activities, including antibacterial, antifungal, antiviral, antiproliferative, analgesic and anti-inflammatory properties.[8] 1,2,3-triazine is the least explored one, till date. But, clinically 1,2,3-triazine derivatives are more acceptable because of potent efficacy and minimal side effect.[9]

Thiophene and its derivatives have been widely distributed in naturally occurring compounds and are employed to treat different health hazards. Thiophene derivatives are responsible for various biological activities such as anti-inflammatory,[10] antipyretic,[11] anti-hypotensive[12], anti-convulsant,[13] anti-viral,[14] antitumor,[15] fungicidal,[16] herbicidal,[17] anti-microbial[18] activities, and act as a plant-growth regulator.[19] Some of the thiophene derivatives exhibited divergences in anti-diabetic and anti-inflammatory activities.[20]

In light of this we planned to synthesize a series of new 1,2,3-triazines carrying thiophene moieties in the hope of obtaining new products of superior biological activity such as antitubercular and anticancer activity.

MATERIALS AND METHODS

All melting points were taken on an Electrothermal IA 9100 series digital melting point apparatus. IR (KBr) were recorded on Perkin-Elmer FT-IR RX-II, H^1 NMR spectra were recorded on brucker AMX 400. Elemental analyses were within ± 0.4% of their calculated values.

SYNTHESIS OF THE COMPOUNDS

Syntheses of 2-amino-3-N-substituted carboxamido-4,5- disubstituted thiophenes (ADVJS - 1 a – 1 l)

A mixture of required active methylenic ketone, substituted cyano acetanilide/s, ammonium acetate and glacial acetic acid (2ml) in benzene (80ml) was refluxed for 10hrs in a Dean stark apparatus with the arrangement of water separation. The reaction mixture was cooled, diluted with benzene and washed successively with water, 10% sodium carbonate solution and dried over anhydrous sodium sulphate. The solvent was removed under vacuum. Later, the crude intermediate was stirred with sulphur in ethanol with the addition of diethylamine drop wise for 1 hr at 45-50^{0}C, chilled overnight and the solid obtained was filtered washed with ethanol to yield yellow crystalline products (**ADVJS -1 a- 1l**).

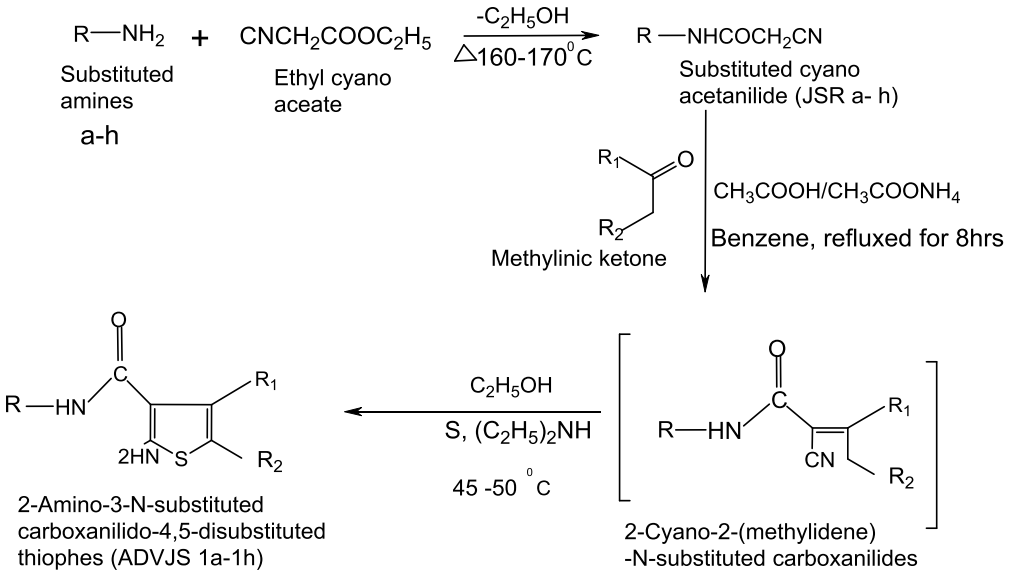

Figure No.1 Syntheses of 2-amino-3-N-substituted carboxamido-4,5- disubstituted thiophenes (ADVJS -1 a – 1 l).

Reaction: Where: R = p-anisyl, o-anisyl, m-anisyl,

R_1, R_2 = -CH_3, - $(CH_2)_3$, - $(CH_2)_4$, - $(CH_2)_5$

Synthesis of bicyclic/tricyclic thieno-1,2,3-triazin-4-ones(ADVJS - 2 a -2 l)

A mixture of the appropriate parent compound (ADVJS -1a-1l) in 30 ml of glacial acetic acid was warmed until the compound was dissolved. Cooled the mixture to room temperature, 20ml of Conc. HCl was added and cooled to a temp. below 5^{0}C. Then to the above mixture an ice cold solution of $NaNO_2$ (0.03 mole) in water (25ml) was added drop wise with constant stirring. Temperature was maintained below 5^{0}C. The product obtained was filtered, dried and washed with methanol to get the desired pure thieno-1,2,3-triazin-4-ones.

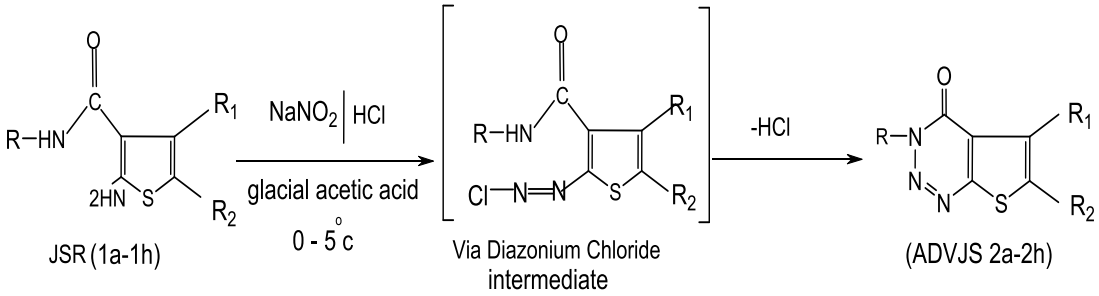

Figure No.2: Synthesis of bicyclic/tricyclic thieno-1,2,3-triazin-4-ones (ADVJS - 2 a-2 l).

Where: R = p-anisyl, o-anisyl, m-anisyl

R_1, R_2 = $-CH_3$, - $(CH_2)_3$, - $(CH_2)_4$, - $(CH_2)_5$

Spectral data of the synthesized compounds

3-N-p-anisyl -5,6- pentamethylene thieno[2,3-d][1,2,3]-triazin-4-(3H)-one: ADVJS2d

IR Cm^{-1}:3404.47 (-NH-st); 1581 (-NH- bend); 2931 (Ali-CH); 1633 (C=O); 1521 (C=C); 752 (C-S).

2-Amino-3-N-(4-methoxy phenyl)–5,6-pentamethylene thiophene ADVJS1d

H^1NMR - $CDCl_3$ showed signals at δ (ppm) = 8.52(s, 1H, -NH, g); 7.53 (d, 2H, Ar-H, h,k); 6.84 (d, 2H, Ar-H, i,j); 5.36 (s, 2H, NH_2, f); 3.78 (s, 3H, $-OCH_3$); 2.81(t, 2H, $-CH_2$-,e); 2.61 (t, 2H, $-CH_2$-, a); 1.83 (s, 2H, $-CH_2$-, d); 1.65 (t, 4H,$-CH_2$,b,c).

3-N-o-anisyl -5,6- pentamethylene thieno[2,3-d][1,2,3]-triazin-4-(3H)-one: ADVJS2h

IR 3054.82cm^{-1}(Ar-CH); 2931.91cm^{-1} (Ali-CH); 1688.32cm^{-1} (C=O); 1562.5cm^{-1} (C=C); 1238.70cm^{-1} (C-O); 818.25cm^{-1} (C-N); 710.08cm^{-1} (C-S).

2-Amino-3-N-(3-methoxy phenyl)–5,6-dimethyl thiophene ADVJS 1i

H^1 NMR- in $CDCl_3$ showed signals at δ (ppm) = 8.44(d, 1H, Ar-H, e); 8.29 (s, 1H,-NH, d); 7.02(m, 2H, Ar-H, f,h); 6.89(d, 1H, Ar-H, g); 5.95 (br, 2H, NH_2, c); 3.89 (s, 3H, $-OCH_3$); 2.33(s, 3H, $-CH_3$, b); 2.20 (s, 3H, $-CH_3$, a).

2-Amino-3-N-(3-methoxy phenyl)–5,6-trimethylene thiophene ADVJS 1j

H^1 NMR - $CDCl_3$ showed signals at δ (ppm) =7.50 (d, 1H, Ar-H, c); 7.34 (d, 1H, -Ar-H, f); 7.21(t, 1H,-Ar-H, d); 7.10(t, 1H, -Ar-H, e); 3.91 (s, 3H, $-OCH_3$); 2.65 (s, 6H, CH_3, a,b).

3-N-p-anisyl -5,6-dimethyl thieno[2,3-d][1,2,3]-triazin-4-(3H)-one: ADVJS 2a

H^1 NMR- $CDCl_3$ showed signals at δ (ppm) = 7.50 (d,2H, Ar-H, c,f); 7.04 (d, 2H,- Ar-H, d,e); 3.87 (s, 3H, $-OCH_3$); 2.55 (s, 6H, CH_3, a,b).

3-N-o-anisyl -5,6-tetramethylene thieno[2,3-d][1,2,3]-triazin-4-(3H)-one: ADVJS 2g

H^1 NMR-$CDCl_3$ showed signals at δ (ppm) = 7.49 (m, 1H, Ar-H, e); 7.37 (d, 1H,- Ar-H, h); 7.10(m, 2H, Ar-H, f,g); 3.81 (s, 3H, -OCH_3); 3.07(d, 2H, -CH_2-, d); 2.91 (t, 2H, -CH_2-, a); 1.90 (m, 4H, -CH_2-, b,c).

BIOLOGICAL ACTIVITY

In Vitro anti-tubercular activity evaluation[21]

The anti-tubercular activity was assessed against *Mycobacteria Tuberculosis* H37Rv using the tube dilution method. This methodology is nontoxic, uses a thermally- stable reagent. All the synthesized compounds were dissolved, separately, in dimethyl sulphoxide(DMSO) to prepare a stock solution containing 1000 µg/mL. The successive concentrations like 50, 100, 200 and 500 µg/mL so on were prepared in a similar manner up to 6 dilutions. A sweep of *Mycobacterial tuberculosis* H37Rv strain culture was discharged with the help of 22 S.G.W. nichrome wire loop with a 3mm external diameter, into a sterile distilled bijou bottle containing six 3mm glass beads and 4 ml distilled water.

All the newly synthesized 1,2,3-Triazenes, were assayed *in vitro* for anti tubercular activity against *Mycobacteria Tuberculosis* H37Rv using the tube dilution method, using isoniazid and rifampicin as a reference standard. This methodology is nontoxic, uses a thermally stable reagent. All the synthesized compounds were dissolved, 10 mg of each, separately in dimethyl sulphoxide to prepare a stock solution containing 1000 µg/mL. The successive concentrations like 10, 20, 40,60,100 and 150 µg/mL were prepared up to 6 dilutions. A sweep of *Mycobacterial tuberculosis* H37Rv strain culture was discharged with the help of nichrome wire loop with a 3mm external diameter, into a sterile distilled bijou bottle containing six 3mm glass beads and 4 ml distilled water. The bottle was shaken with the help of a mechanical shaker for 2 min. Using nichrome wire loopful of the suspension was inoculated on the surface of each of Lowenstein-Jensen medium containing test compounds. Lowenstein-Jensen medium containing Isoniazide standard drug as well as control was also inoculated with *Mycobacterial tuberculosis* H37Rv strain. The medium inoculated were incubated at 37°C for six weeks. The presence or absence of growth of organism was observed after six weeks. The MIC (minimal inhibitory concentration) was defined as the lowest drug concentration, which prevented the microbial growth.

IN-VITRO ANTI-CANCER ACTIVITY[22]

Procurement of cancer cell lines

The HT-29 (human colorectal adenocarcinoma) cell line was initially procured from the National Centre for Cell Sciences (NCCS), Pune, India, and maintained in DMEM. The cell line was cultured in 25 cm^2 tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate and antibiotic solution containing: penicillin (100 U/ml), streptomycin (100 µg/ml). Cultured cell line was kept at 37°C in a humidified 5% CO_2 incubator (VWR, USA). Two-day-old confluent monolayer of cells were trypsinized and the cells were suspended in 10% growth medium, 100 µl cell suspension (5×10^4 cells/well) was seeded in 96-well tissue culture plate and incubated at 37°C in a humidified 5% CO_2 incubator for 24 h. The viability of cells was evaluated by direct observation of cells by Inverted phase contrast microscope and followed by MTT assay method.

MTT Viability assay

The cytotoxic potential of the most active derivatives (as mentioned in the above section) was evaluated by MTT (3,4,5-dimethylthiazol-2-yl)-2-5-diphenyltetrazolium bromide) assay which is based on the reduction of the yellow colored water-soluble tetrazolium dye MTT by the mitochondrial lactate dehydrogenase formed by the live cells to the formazan crystals, which display purple color upon dissolution into the suitable solvent. The intensity of the purple color is directly proportional to the number of viable cells and can be measured by spectrophotometer at 570 nm. The HT-29 cells were treated with different concentrations of these derivatives (12.5, 25, 50 and 100 µg/ml) for 24 h and observed for anticancer activity by MTT assay using ELISA reader. Anticancer efficacy of selected synthesized compounds was measured in terms of percentage of growth inhibition by using following formula:

% of inhibition = [Absorbance of sample/Absorbance of control] X 100

RESULTS AND DISCUSSION

Synthesis

The synthesis of Syntheses of 2-amino-3-N-substituted carboxamido-4,5- disubstituted thiophenes (ADVJS -1 a – 1 l) and bicyclic/tricyclic thieno-1,2,3-triazin-4-ones(ADVJS - 2a - 2l) is shown in [Figure 1 and 2]. All synthesized compounds subjected for physical melting point determination data shown in [Table 1] and the structure of selected synthesized compounds was confirmed by IR and ^1H NMR spectral data.

Anti-tubercular activity

The results of anti-tubercular activity are shown in table No.2. The synthesized derivative of 1,2,3-triazines with dose range from 10 to 150 µg/ml tested against *M. tuberculosis* $H_{37}Rv$. ADVJS 2a (3-N-p-anisyl -5,6-dimethyl thieno [2,3-d][1,2,3]-triazin-4-(3H)-one) and ADVJS2d (3-N-p-anisyl -5,6- pentamethylene thieno [2,3-d][1,2,3]-triazin-4-(3H)-one) with p-tolyl substituent and showed better activity over other synthesized compounds.

Anticancer activity

The results of anticancer activity are shown in table No.3. The 1,2,3-triazine synthesized derivative at concentration 12.5, 25, 50 and 100 µg/ml were tested against HT-29 (human colorectal adenocarcinoma) cell line by MTT viability assay. The newly synthesized triazines ADVJS -2a to 2d with p- tolyl substituent at R showed better anti cancer activity followed by the compounds ADVJS -2e to 2h with o- tolyl substituent at R and ADVJS -2i to 2l with m-tolyl substituent at R (Table No.3).

CONCLUSION

This study indicates that the anti-tubercular and anti-cancer activities of the synthesized 1,2,3- triazines compounds can be attributed to the presence of o-tolyl substituent in triazines. The findings of this work should be helpful to medicinal chemists involved in further drug development in this field.

ACKNOWLEDGMENT

The authors are thankful to Rajiv Gandhi University of Health Sciences, Bangalore, Karnataka for providing all necessary facilities to carry out the research work.

Table No.1 Physical data of the new bicyclic/tricyclic Thieno 1,2,3 – triazine- 4- ones (ADVJS 2a-2l).

Sr. No.	Comp.No.	R	R_1,R_2	M.P.(^OC)	
1	ADVJS-2a	p-anisyl	$-CH_3$	166	**Triazines were Washed only With methanol to get pure compounds**
2	ADVJS-2b	p-anisyl	$-(CH_2)_3-$	138	
3	ADVJS-2c	p-anisyl	$-(CH_2)_4-$	160	
4	ADVJS-2d	p-anisyl	$-(CH_2)_5-$	143	
5	ADVJS -2e	o-anisyl	$-CH_3$	110	
6	ADVJS -2f	o-anisyl	$-(CH_2)_3-$	178	
7	ADVJS -2g	o-anisyl	$-(CH_2)_4-$	140	
8	ADVJS -2h	o-anisyl	$-(CH_2)_5-$	152	
9	ADVJS -2i	m-anisyl	$-CH_3$	108	
10	ADVJS -2j	m-anisyl	$-(CH_2)_3-$	140	
11	ADVJS -2k	m-anisyl	$-(CH_2)_4-$	126	
12	ADVJS -2l	m-anisyl	$-(CH_2)_5-$	136	

Table No. 2: Anti-tubercular activity of synthesized 1,2,3-Triazines derivatives.

Sl.No	Compound	MIC values (µg/ml) of *Mycobacterium tuberculosis* $H_{37}Rv$
01	ADVJS -2a	80
02	ADVJS -2b	60
03	ADVJS -2c	**20**
04	ADVJS -2d	80
05	ADVJS -2e	150
06	ADVJS -2f	100
07	ADVJS -2g	100
08	ADVJS -2h	120
09	ADVJS -2i	150
10	ADVJS -2j	150
11	ADVJS -2k	100
12	ADVJS -2l	120
	Isoniazid	0.25

Table No. 3: Anti-cancer activity of synthesized 1,2,3-triazines derivatives.

Sample Code	Concentration (µG/ML)	Absorbance (540 NM)	Percentage of Viability
Control	0.6495		
ADVJS -2a	12.5	0.4886	75.22±0.78
	25	0.4572	70.39±0.97
	50	0.3956	60.90±0.393
	100	0.312	48.03±0.156
ADVJS -2b	12.5	0.4943	76.10±0.596
	25	0.4017	61.84±0.511
	50	0.3602	55.45±0.466
	100	0.3193	49.16±0.438
ADVJS -2c	12.5	0.4989	76.81±0.332
	25	0.4836	74.45±0.485
	50	0.4043	62.24±0.831
	100	0.3035	46.72±0.256
ADVJS -2d	12.5	0.4235	65.20±0.031
	25	0.3569	54.94±0.615
	50	0.3083	47.46±0.825
	100	0.2393	36.84±0.259
ADVJS -2e	12.5	0.4137	63.69±0.01
	25	0.3178	48.92±0.61
	50	0.30854	47.50±0.54
	100	0.2863	44.08±0.16
ADVJS -2f	12.5	0.4411	67.91±0.98
	25	0.4308	66.32±0.46
	50	0.4217	59.01±0.66
	100	0.3833	43.40±0.74
ADVJS -2g	12.5	0.4525	69.68±0.61
	25	0.4232	65.15±0.37
	50	0.3695	56.88±0.53

	100	0.3601	44.59±0.24
ADVJS -2h	12.5	0.4117	63.38±0.09
	25	0.3886	59.83±0.39
	50	0.3787	58.30±0.95
	100	0.3038	46.77±0.19
ADVJS -2i	12.5	0.3412	52.53±0.75
	25	0.3064	47.17±0.98
	50	0.2819	43.40±0.74
	100	0.2692	41.44±0.71
ADVJS -2j	12.5	0.3867	59.53±0.62
	25	0.3401	52.36±0.64
	50	0.2948	45.38±0.06
	100	0.2877	44.29±0.12
ADVJS -2k	12.5	0.4089	62.95±0.01
	25	0.3763	57.93±0.45
	50	0.3622	55.76±0.38
	100	0.3026	46.58±0.44
ADVJS -2l	12.5	0.2984	45.93±0.31
	25	0.2878	44.31±0.85
	50	0.2687	41.37±0.48
	100	0.2597	39.98±0.35

REFERENCES

1. Shingalapur RV, Hosamani KM, Keri RS Synthesis and evaluation of in vitro antimicrobial and antitubercular activity of 2-styryl benzimidazoles. Eur J Med Chem., 2009; 44: 4244–4248.
2. WHO annual TB report 2017. http://apps.who.int/iris/bitstream/10665/254762, accessed 24 July 2017.
3. Patel RV, Patel PK, Kumari P, Rajani DP, Chikhalia KH Synthesis of benzimidazolyl-1,3,4-oxadiazol-2ylthio-*N*-phenyl(benzothiazolyl) acetamides as antibacterial, antifungal and antituberculosis agents. Eur J Med Chem., 2012; 53: 41–51.
4. Pogorzelska A, Sławiński J, Żołnowska B, Szafrański K, Kawiak K, Chojnacki J, Ulenberg S, Zielińska J, Bączek T Novel 2-(2-alkylthiobenzenesulfonyl)-3-(phenylprop-2-ynylidene amino) guanidine derivatives as potent anticancer agents—synthesis, molecular structure, QSAR studies and metabolic stability. Eur J Med Chem., 2017; 44: 3350-3355.
5. Slawinski J, Szafranski K, Pogorzelsk A, Zolnowska B, Kawiak A, Macur K, Belka M, Baczek T. Novel 2-benzylthio-5-(1,3,4-oxadiazol-2-yl) benzene sulfonamide with anticancer activity synthesis, QSAR study and metabolic stability. Eur J Med Chem., 2017; 132: 236-248.

6. Bairi KE, Ouzir M, Agnieszka N, Khalki L. Anticancer potential of *Trigonella foenum graecum*: cellular and molecular targets. Biomed Pharmacother, 2017; 90: 479–491.
7. Ghorab MM, Ragab FA, Heiba HI, Soliman AM. Design and synthesis of some novel 4-chloro-*N*-(4-(1-(2-(2-cyanoacetyl)hydrazono)ethyl)phenyl) benzene sulfonamide derivatives as anticancer and radiosensitizing agents. Eur J Med Chem., 2016; 124: 946-958.
8. El Rashedy AA, Aboul-Enein HY. Benzimidazole derivatives as potential anticancer agents. Mini Rev Med Chem., 2013; 13(2): 399-407.
9. Stella Cascioferro, Barbara Parrino, Virginia Spano, Anna Carbone, et al., Synthesis and antitumor activity of 1,2,3-trizenes and their benzo and hetero-fused derivatives. European Journal of Medicinal Chemistry, 2017; 142: 74-86.
10. D. Srinivasa Rao, G. V. Pavan Kumar, B. Pooja, G. Harika, Y. Anil Kumar and G. Sadasiva Rao. An extensive review on 1,2,3 and 1,2,4-triazines scaffold-valuable lead molecules with potent and diverse Pharmacological activities. Der Chemica Sinica, 2016; 7(2): 101-130.
11. Molvi K.I, Mansuri M, Sudarsanam V, Patel M.M, Andrabi S.M, Haque N. Synthesis, anti-inflammatory, analgesic and antioxidant activities of some tetrasubstituted thiophenes. J. Enzyme Inhib. Med. Chem., 2008; 23: 829–838.
12. Gouda M.A, Eldien H.F, Girges M.M, Berghot M.A. Synthesis and antitumor evaluation of thiophene-based azo dyes incorporating pyrazolone moiety. J. Saudi Chem. Soc., 2016; 20: 151–157.
13. Desai N.C, Dodiya A.M, Rajpara K.M, Rupala Y.M. Synthesis and antimicrobial screening of 1,3,4-oxadiazole and clubbed thiophene derivatives. J. Saudi Chem. Soc., 2014; 18: 255–261.
14. Deep A, Narasimhan B, Aggarwal S, Kaushik D, Arun K.S. Thiophene scaffold as prospective central nervous system agent: A review. Cent. Nerv. Syst. Agents Med. Chem., 2016; 16: 58–64.
15. Fathima N, Ziaulla M, Banu A, Panchamukhi S.I, Khazi I.A, Begum N.S. Synthesis, spectroscopy and X-ray crystal structure of 9-methyl-3-thiophen-2-yl-thieno[3,2-*e*] [1,2,4] triazolo [4,3-*c*]pyrimidine-8-carboxylic acid ethyl ester. Am. J. Anal. Chem., 2011; 2: 371 –375.
16. Pulipati L, Yogeeswari P, Sriram D, Kantevari S. Click-based synthesis and antitubercular evaluation of novel dibenzo[*b*,*d*]thiophene-1,2,3-triazoles with piperidine,

piperazine, morpholine and thiomorpholine appendages. Bioorg. Med. Chem. Lett., 2016; 26: 2649–2654.

17. Wang X, Zhu L, Chen S, Xu X, Au C.T, Qiu R. Nickel-catalyzed direct C (sp3)–H arylation of aliphatic amides with thiophenes. Org. Lett., 2015; 17: 5228–5231.
18. Benachenhou F, Mesli A, Guilard R. Synthesis of Schiff bases by aromatic amine condensation with 3,3′-bithiophenes-2,2′ and 4,4′-dicarbaldehydes. Arabian J. Chem., 2013; 6: 313–317.
19. Rani M, Mohamad Y. Synthesis, studies and in vitro antibacterial activity of some 5-(thiophene-2-yl)-phenyl pyrazoline derivatives. J. Saudi Chem. Soc., 2014; 18: 411–417.
20. Gopi C, Dhanaraju M.D. Synthesis, spectroscopy characterization and biological activities of some novel 1-(3-(*N*,*N*-dimethylamino)-1-(5-substituted thiophene-2-yl) propylidene semicarbazone Mannich base derivatives. Beni-Suef Univ. J. Basic Appl. Sci., 2018; 7(2): 291-298.
21. B. Watt, A. Rayner, G. Harris, Chapter 41. In: G. J. Collee, M. Fraser, A. Simmons, Mackie, McCartney, Practical Medical Microbiology. New York: Churchill Livingstone, 1996; 331-335.
22. Mosmann, T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J. Immunol. Methods, 1983; 65: 55–63.