European Journal of Medicinal Chemistry 45 (2010) 4365–4369

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Short communication

Synthesis of some 3-substituted amino-4,5-tetramethylene thieno[2,3-*d*] [1,2,3]-triazin-4(3*H*)-ones as potential antimicrobial agents

Janardhanan Saravanan*[,1], Shamanna Mohan [1], Jay Jyoti Roy [1]

Department of Pharmaceutical Chemistry, P.E.S College of Pharmacy, 50 Ft Road. Hanumanthanagar, Bangalore -560 050, Karnataka, India.

ARTICLE INFO

Article history:
Received 21 March 2010
Received in revised form
26 May 2010
Accepted 27 May 2010
Available online 2 June 2010

Keywords:
Synthesis
Gewald reaction
Thiophenes
1,2,3-Triazine-4-ones
Diazotization
Antimicrobial evaluation

ABSTRACT

A series of 3-Substituted amino-4,5-tetramethylene thieno[2,3-*d*] [1,2,3]-triazine-4(3*H*)-ones have been synthesized and characterized by UV,IR, 1H NMR, elemental and mass spectral analysis. The title compounds were evaluated for their antimicrobial activity by agar diffusion method against four bacteria and three fungi using Ampicillin and Miconazole nitrate as standards. The compounds **VIIIa**, **IXa**, **Xa** and **XIa** showed an antimicrobial efficacy considerably greater than the compounds **Ia** to **VIIa** with −H, phenyl and electron donating (activating) groups like methyl, ethyl and tolyl substitutions at **R**, suggesting that lipophillic groups like chloro, fluoro substitution on the phenyl ring plays an important role in enhancing the antimicrobial properties of this class of compounds.

From the screening results it can be concluded that the compounds having the lipophillic groups like chlorophenyl and fluorophenyl groups at **R** exhibited appreciable antimicrobial activities. Whereas, the compounds are having −H, phenyl and electron donating (activating) groups like methyl, ethyl and tolyl substituents at **R** were less active against all the organisms used.

1. Introduction

Resistance to antimicrobial agents (AMR) by pathogenic bacteria towards available antibiotics is rapidly becoming a major worldwide problem; the design of new compounds to deal with resistant bacteria has become one of the most important areas of antibacterial research today. In addition, primary and opportunistic fungal infections continue to increase rapidly because of the increased number of immune compromised patients. As known, not only biochemical similarity of the human cell and fungi forms a handicap for selective activity, but also the easily gained resistance is the main problem encountered in developing safe and efficient antifungal agents.

Literature survey reveals that thiophenes such as substituted thiophenes and condensed thiophenes are interesting compounds being studied in medicine and they are reported to possess an array of useful biological activities such as antibacterial [1,2], antifungal [3–5], antiviral [6,7], antiprotozoal [8,9] and herbicidal [10,11].

1,2,3-triazine [12–17] represents an important nitrogen heterocycle containing a 6 membered ring with 3 nitrogen atoms. Theoretically, three triazines are possible of them 1,2,3-triazine is scarcely explored.

The aim of the present study is therefore to synthesize some new condensed thiophenes like thieno-1,2,3-triazine-4-ones and to investigate their efficacy on pathogenic strains of Gram-positive, Gram-negative bacteria and fungi.

2. Chemistry

The title compounds were synthesized with an aim of exploring their antimicrobial value.

A large number of medicinal compounds which have been discovered belong to a major class of heterocycles containing Nitrogen and Sulphur. The versatile synthetic applicability and biological activity of these heterocycles has helped the medicinal chemists to plan, organize and implement new approaches towards the discovery of novel drugs.

Thiophenes and its derivatives are an important class of heterocyclic compounds, particularly, 2-amino substituted thiophenes reported to possess a wide spectrum of biological properties such as antibacterial, antifungal, antiviral, analgesic, anti-inflammatory, antioxidant, antitumor and So on. Thiophene containing β-lactam antibiotics like Ticarcillin, Cefoxitin, Cephalothin and Cephalorodine have shown good antibacterial activity.

* Corresponding author. Tel.: +91 80 2660 0741, +91 9880118910; fax: +91 80 2650 6928.
E-mail addresses: drjayes@gmail.com (J. Saravanan), smohan100@hotmail.com (S. Mohan), jayroy06@gmail.com (J.J. Roy).
[1] Tel.: +91 80 2660 0741, +919448870387, +919163621164; fax: +91 80 2660 0113.

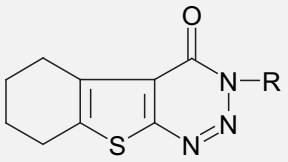
0223-5234/$ – see front matter © 2010 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2010.05.061

Similarly antifungal Ticonazole and Sertaconazole also contain thiophene.

1,2,3-triazines represent a widely used lead structure with multitude of interesting applications in numerous fields. Diazonium ion condensation with an adjacent nucleophilic function to form a 5 or 6 membered ring has proved valuable for synthesizing various nitrogen heterocycles. Diazotization of an amino group bearing aryl and heteroaryl carboxamides at the ortho position is often used for the construction of 1,2,3-triazine rings [18].

The synthetic route employed for the synthesis of the title compounds is outlined in Fig. 1. As large numbers of 2-amino thiophene-3-carboxylic esters were reported [19,20], it was thought that the conversion of esters to their corresponding anilides could be an attractive method for the synthesis of 2-amino-thiophen-3-carboxanilides. However, these 2-aminothiophen-3-carboxylic esters when made to react with nitrogen nucleophiles, such as amines could not undergo intermolecular condensation. This is not unexpected in view of the fact that the presence of ortho amine function leads to the decrease in the deficiency of electron density at the carbonyl group of the ester function. In general, it appears that thiophene amino esters are less reactive than anthranilic acid esters. The starting compounds 2-amino-3-(N-substituted carboxamido)-4,5-tetramethylene thiophenes **I**–**XII** were synthesized involving three steps by the adaptation of well known and versatile Gewald reaction [21]. Later, the compounds **I**–**XII** were diazotized to yield a series of 3 - Substituted amino-5,6-Tetramethylene thieno[2,3-*d*] [1,2,3]-triazin-4(3*H*)-ones **Ia**–**XIIa.** In this reaction, the starting compounds 2-amino-3-(N-substituted carboxamido)-4,5-tetramethylene thiophenes (**I**–**XII**) reacts with $NaNO_2$ in presence of HCl to give respective triazine-4-ones. The physical properties are reported in the Table 1.

Table 1



Ia–**XIIa**

Comp no.	R	M.P °C	% Yield	R_f (Solvent $CHCl_3$:MeOHl) (70:30)
Ia	-H	168–169	52	0.48
IIa	Methyl	103–105	46	0.56
IIIa	Ethyl	131–134	49	0.62
IVa	Phenyl	112–114	47	0.71
Va	2-Methylphenyl	121–122	51	0.59
VIa	3-Methylphenyl	138–140	64	0.80
VIIa	4-Methylphenyl	175–176	71	0.63
VIIIa	4-Fluorophenyl	154–156	67	0.41
IX a	2-Chlorophenyl	145–146	76	0.77
Xa	3-Chlorophenyl	158–160	71	0.35
XIa	4-Chlorophenyl	110–112	62	0.72
XIIa	3-Chloro-4-fluorophenyl	125–126	79	0.84

3. Biological assay

3.1. Antimicrobial activity

The standard strains were procured from the American Type Culture Collection (ATCC) and Gene Bank, Institute of Microbial Technology, Chandigarh, India. Antibacterial [22] and antifungal [23] activity of the title compounds were carried out by agar cup diffusion method at a concentration of 50 μg/ml using four standard bacterial strains viz; *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Salmonella typhi* and three fungi viz; *Aspergillus niger, Candida albicans & Cryptococcus neoformans.* The MIC of compounds was determined by tube dilution method. The result of the MIC of the compound is illustrated in the Table 2.

The zone of inhibition was measured in mm. The test compounds were dissolved in DMF to get the concentration of 50 μg/ml. The fresh sub culture of bacteria in isotonic sodium chloride solution was added to the sterile assay medium at 40–45 °C and mixed well. The medium was poured into each of the petridishes. The test solution of different derivatives was added to the previously marked wells and the media was allowed to stand for 5 min. The petridishes were covered and set aside for 1 h and then incubated at 37 °C for 24 h and the zones of inhibition were measured and the average of three readings was calculated. The activity was compared with Ampicillin. The antifungal activity was carried in the same way at28 °C for 48 h and compared with Miconazole nitrate (50 μg/ml) as standard. The readings are tabulated in Tables 3 and 4 respectively.

4. Results and discussion

Very few condensed 1,2,3-triazin-4-ones have been reported with antibacterial and antifungal activity. Diazonium ion condensation with an adjacent nucleophilic function has proved valuable for the construction of the desired 6 membered 1,2,3-triazines as our title compounds.

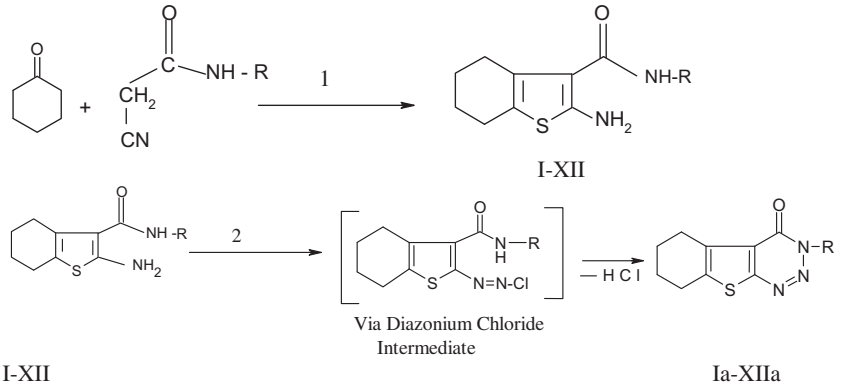


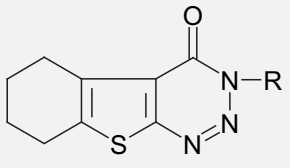
Fig. 1. Reagents and Conditions: (1) C_6H_{12}, CH_3COOH, CH_3COONH_4, S, C_2H_5OH,$(C_2H_5)_2$ NH. (2) $NaNO_2$/HCl, CH_3COOH, 0–5 °C. R=–H, methyl, ethyl, phenyl, 2-methylphenyl, 3-methylphenyl, 4-methylphenyl, 4-fluorophenyl, 2-chlorophenyl, 3-chlorophenyl, 4-chlorophenyl, 3-chloro-4-fluorophenyl.

Table 2
Minimum inhibitory concentration of the titled compounds and Ampicillin, Miconazole nitrate as a standard in μg/ml concentrtation.

Compd No.	Antimicrobial activity				Antifungal activity		
	Gm +Ve		Gm −Ve				
	Sa	*Se*	*E.coli*	*St*	*An*	*Ca*	*Cn*
Ia	37.5	37.5	50	37.5	37.5	50	125
IIa	37.5	37.5	50	37.5	37.5	50	125
IIIa	37.5	37.5	50	37.5	37.5	50	125
IVa	25	25	37.5	37.5	37.5	50	125
Va	25	25	37.5	25	25	37.5	75
VIa	25	25	37.5	25	25	37.5	75
VIIa	25	25	37.5	25	25	37.5	75
VIIIa	6.75	6.75	12.5	12.5	12.5	37.5	75
IXa	12.5	12.5	25	12.5	12.5	25	50
Xa	12.5	12.5	25	12.5	12.5	25	50
XIa	12.5	12.5	25	12.5	12.5	25	37.5
XIIa	12.5	12.5	25	12.5	25	37.5	50
Ampicillin	0.5	1.56	4	6.25	–	–	–
Miconazole nitrate					10	12.5	20

The formation of the starting compound I was confirmed by its IR spectra where the two strong stretching band at 3180 cm^{-1}–3350 cm^{-1} are due to the presence of primary amide group and two prominent peaks between 3300 and 3500 cm^{-1} of primary amine and stretching band at 1666 cm^{-1} accounting for amide carbonyl group. The compound II illustrates only one amide peak at 3320 cm^{-1} due to the presence of 2° amide and an additional stretching band at 2900 cm^{-1} and a bending absorption at 1360 cm^{-1} for the methyl group present in the compound. The compound III has additional band at 1455 cm^{-1} accounting for the methylene group Compared to the compound II. The formation of remaining compounds (No. IV to XII) were confirmed by the presence of strong stretching band at 3050–3010 cm^{-1} due to aromatic =C–H and a pair stretching band at 1600 and 1490 cm^{-1} accounting for C=C.

Table 3



Ia–XIIa

Comp no. R		Antibacterial activity Zone of Inhibition in mm			
		Sa	*Se*	*Ec*	*St*
IIa	Methyl	16	18	12	14
IIIa	Ethyl	18	16	16	16
IVa	Phenyl	20	17	12	12
Va	2-Methylphenyl	20	18	14	12
VIa	3-Methylphenyl	24	20	14	14
VIIa	4-Methylphenyl	28	28	20	18
VIIIa	4-Fluorophenyl	32	32	26	24
IX a	2-Chlorophenyl	30	30	24	20
Xa	3-Chlorophenyl	32	30	26	24
XIa	4-Chlorophenyl	34	32	26	26
XIIa	3-Chloro-4-fluorophenyl	26	24	24	20
	Ampicillin	40	36	28	26

Sa = *Staphylococcus aureus*, *Se* = *Staphylococcus epidermidis*, *Ec* = *Escherichia coli*, *St* = *Salmonella typhi*.
Zone of inhibition in mm at a concentration of 50 μg/ml.

Table 4

Comp no.	R	Antifungal activity Zone of Inhibition in mm		
		An	*Ca*	*Cn*
Ia	–H	04	03	NA
IIa	Methyl	06	04	NA
IIIa	Ethyl	06	05	NA
IVa	Phenyl	06	04	NA
Va	2-Methylphenyl	06	04	NA
VIa	3-Methylphenyl	08	04	06
VIIa	4-Methylphenyl	08	05	05
VIIIa	4-Fluorophenyl	10	08	08
IXa	2-Chlorophenyl	14	08	09
Xa	3-Chlorophenyl	13	08	08
XIa	4-Chlorophenyl	16	10	10
XIIa	3-Chloro-4-fluorophenyl	10	07	08
	Miconazole nitrate	16	12	14

An = *Aspergillus niger*, *Ca* = *Candida albicans* & *Cn* = *Cryptococcus neoformans*.
Zone of inhibition in mm at a concentration of 50 μg/ml.

The formation of the thienotriazine-4-ones was confirmed by their preliminary observations including M.P, R_f values, UV absorption maxima and IR spectra, compared to their starting materials.

The UV absorption spectra of the new thienotriazines exhibited a bathochromic shift (20–25 nm) from their starting materials due to aryl cyclization. The formation of the thienotriazines were also confirmed by the shift of IR peaks between 1630 and 1640 cm^{-1} as seen in the starting materials to 1690–1700 cm^{-1} in the final compounds indicating the aryl cyclization due to the cyclic keto group and absence of the prominent peaks between 3300 and 3460 cm^{-1} of $-NH_2$ in triazines compared to their starting thiophenes. The ^1H NMR of the compounds adds to the proof for the formation of the new thienotriazines. The ^1H NMR of the compound **Ia** show the singlet peak at 8.1 and the absence of the singlet peak at 4.9 due to the primary amino group add to the proof for the formation of the new thienotriazines. In the ^1H NMR of the compound **IIa** showed the singlet peak at 2.74 accounts for the presence of methyl group. For the remaining compounds (Nos. **IVa**–**XIIa**) the attachment of Un/substituted phenyl groups was confirmed by the presence of the corresponding peaks between 6.5 and 8.0.

The results of antimicrobial study of the title compounds were illustrated in the Tables 2–4.

From the antimicrobial activity results of the title compounds it was concluded that the compounds with liphophilic substitutions at the phenyl ring were more effective against all the organisms used than the unsubstituted phenyl ring. Particularly the compound **VIIIa** with 4-fluorophenyl substitution exhibited excellent inhibition at MIC 6.75 μg/mL against tested gram +ve bacteria and 12.5 μg/mL against gram –ve bacteria. In case of the antifungal activity the compound having 4-chlorophenyl substitution at R as in **XIa** shown promising activity.

The MIC study also confirms that the potency of the title compounds are based on the substituents attached to the phenyl group. Finally among the compounds screened, the compound **XIa** with 4-chlorophenyl substituent at **R** on triazin–4-one nucleus was potent against all types of bacteria and fungi employed.

5. Experimental

Analytical TLC was performed on Silica plates- GF254 (Merck) with visualization by UV or iodine vapors. Melting points were determined in open capillaries on a Thermonic Melting point apparatus and are uncorrected. The IR spectra (KBr, cm^{-1}) were run

on Perkin Elmer FTIR Spectrophotometer. ^{1}H NMR (in $CDCl_3$/DMSO-d_6) spectra were recorded using Bruker AMX-400 with TMS as internal standard. MS spectra were recorded on (AMD-604). Elemental analyses were performed on Carlo Erba 1108 elemental analyzer and were within ±0.4% of theoretical values. The result of elemental analysis is tabulated in the Table 5. All the chemicals used were of analytical grade.

5.1. General procedure for the syntheses of 2-amino–3-N-substituted carboxamido/carboxanilido–4,5-tetra methylene thiophenes (*I*–*XII*)

A mixture of appropriate active methylenic ketone (0.04 mol), substituted cyano acetamide/acetanilide (0.04 mol), ammonium acetate (2 g) and glacial acetic acid (2 ml) in cyclohexane (80 ml) was refluxed for 10 h in a Dean stark apparatus with an arrangement for water separation. The reaction mixture was cooled, diluted with cyclohexane and washed successively with water, 10% aqueous sodium carbonate solution, and dried over anhydrous sodium sulphate. The solvent was removed under vacuum. The crude alpha (N-substituted carboxamido/carboxanilido) acetonitrile derivative thus obtained was employed directly for further reaction.

To a mixture of the above crude intermediate and sulphur (0.04 mol) in ethanol (40 ml) was added diethylamine (4.0 ml) drop wise with stirring. The mixture was stirred for 1 h at 45–50 °C, chilled overnight and the solid obtained was filtered washed with ethanol to yield yellow crystalline solids. Recrystallized from suitable solvents. Yield – 45–50%

5.2. General method for the syntheses of 4,5-tetramethylene thieno [2,3-d][1,2,3]-triazin-4(3H)-ones (*Ia*–*XIIa*)

A mixture of the corresponding 2-amino-3-*N*-(substituted carboxamido/carboxanilido)-4,5-substituted thiophenes (**I**–**XII**) (0.01 mol) in 30 ml of glacial acetic acid was warmed until the starting material dissolved. The mixture was cooled to room temperature, 20 ml of concentrated HCl was added and the reaction mixture was cooled to a temperature below 5 °C. To this mixture an ice cold solution of $NaNO_2$ (0.03 mol) in water (25 ml) was added drop wise with constant stirring. Temperature was maintained below 5 °C. The product separated as bright yellow solid, which was filtered, dried and washed with methanol to obtain pure triazines (**Ia**–**XIIa**).

Table 5
Elemental Analysis of the synthesized compounds (**Ia**–**XIIa**).

Compd No	Mol. Formula	Mol. Wt	Elemental Analysis	
			Theoretical	Found
Ia	$C_9H_9N_3OS$	207	$C = 52.16$; $H = 4.38$; $N = 20.27$; $S = 15.47$	$C = 52.19$; $H = 4.31$; $N = 20.27$; $S = 15.44$
IIa	$C_{10}H_{11}N_3OS$	221	$C = 54.29$; $H = 5.01$; $N = 18.99$; $S = 14.49$	$C = 54.28$; $H = 4.96$; $N = 19.42$; $S = 14.11$
IIIa	$C_{11}H_{13}N_3OS$	235	$C = 56.17$; $H = 5.5$; $N = 17.87$; $S = 13.61$	$C = 56.58$; $H = 5.02$; $N = 18.34$; $S = 13.27$
IVa	$C_{15}H_{13}N_3OS$	283	$C = 63.58$; $H = 4.62$; $N = 14.84$; $S = 11.32$	$C = 63.60$; $H = 4.63$; $N = 14.82$; $S = 11.33$
Va	$C_{16}H_{15}N_3OS$	297	$C = 64.62$; $H = 5.08$; $N = 14.14$; $S = 10.77$	$C = 64.64$; $H = 5.07$; $N = 14.17$; $S = 10.76$
VIa	$C_{16}H_{15}N_3OS$	297	$C = 64.62$; $H = 5.08$; $N = 14.14$; $S = 10.77$	$C = 64.59$; $H = 5.07$; $N = 14.12$; $S = 10.78$
VIIa	$C_{16}H_{15}N_3OS$	297	$C = 64.62$; $H = 5.08$; $N = 14.14$; $S = 10.77$	$C = 64.66$; $H = 5.09$; $N = 14.15$; $S = 10.78$
VIIIa	$C_{15}H_{12}N_3OSF$	301	$C = 59.80$; $H = 4.01$; $N = 13.95$; $S = 10.64$	$C = 59.91$; $H = 4.02$; $N = 13.93$; $S = 10.67$
IXa	$C_{15}H_{12}N_3OSCl$	317.5	$C = 56.69$; $H = 3.81$; $N = 13.22$; $S = 10.07$	$C = 56.56$; $H = 3.80$; $N = 13.26$; $S = 10.09$
Xa	$C_{15}H_{12}N_3OSCl$	317.5	$C = 56.69$; $H = 3.81$; $N = 13.22$; $S = 10.07$	$C = 56.72$; $H = 3.82$; $N = 13.19$; $S = 10.10$
XIa	$C_{15}H_{12}N_3OSCl$	317.5	$C = 56.69$; $H = 3.81$; $N = 13.22$; $S = 10.07$	$C = 56.60$; $H = 3.82$; $N = 13.21$; $S = 10.09$
XIIa	$C_{15}H_{11}N_3OSClF$	335.5	$C = 53.65$; $H = 3.30$; $N = 12.51$; $S = 9.55$	$C = 53.75$; $H = 3.31$; $N = 12.47$; $S = 9.56$

5.2.1. 4,5-Tetramethylene thieno-[2,3-d][1,2,3]-triazin-4(3H)-ones: (*Ia*)

M.P: 168–169 °C yield: 52% IR max cm^{-1} = –SCH 2940, –NH 3300–3200, –CO –1660. NMR: Solvent $CDCl_3$, 8.2(s, 1H, –NH proton), 2.6 (m, 4H, methylenic proton), 1.5 (m, 4H, methylenic proton). MS (%) 207.04 (100%), 208.06 (9.7%), 209.05 (4.5%), 208.04 (1.1%).

5.2.2. 3-N-Methyl-4,5-tetramethylene thieno-[2,3-d][1,2,3]-triazin-4-(3H)-ones:(*IIa*)

M.P: 103–105 °C yield: 46% IR max cm^{-1} = Ar-CH 3024.83: -Ali-CH 2978.54; Arom C=C 1506.46; –CO –1660; (C–N)–746.23 NMR: Solvent -$CDCl_3$: 3.1 (s, 3H, – CH_3), 2.8 (m, 4H, methylenic protons), 1.4 (m, 4H, methylenic protons). MS (%) 221.06 (M^+ 100.0%), 222.07 (11.3%), 223.06 (4.6%), 222.06 (1.9%).

5.2.3. 3-N-Ethyl-4,5-tetramethylene thieno-[2,3-d][1,2,3]-triazin-4-(3H)-ones:(*IIIa*)

M.P: 131–134 °C yield: 49% IR max cm^{-1} = -Ar-CH 3027.53: -Ali-CH 2988.42; Arom C=C 1505.46; – CO –1682; (C–N)–753. 32 NMR: Solvent – $CDCl_3$ 3.0 (q, 2H, -CH_2), 1.3 (t,3H, –CH_3), 2.4 (m,4H, methylenic protons), 1.6 (m,4H, methylenic protons). MS (%) 235.08 (M^+ 100.0%), 236.08(13.3%), 237.07 (4.4%), 237.08 (1.1%), 236.07 (1.1%).

5.2.4. 3-N-Phenyl-4,5-tetramethylene thieno[2,3-d][1,2,3]-triazin-4-(3H)-ones: (*IVa*)

M.P: 112-114 °C yield: 47% IR max cm^{-1} = Ar-CH 3035.23: -Ali-CH 2982.54; Arom C=C 1498. 63; – CO –1692; (C–N) –759.23 NMR: Solvent –$CDCl_3$ 7.25–7.5 (m, 5H, Arom), 2.5 (m,4H, methylenic proton), 1.5 (m,4H, methylenic protons). MS (%) 283.08 (M^+ 100.0%), 284.08(17.7%), 285.07 (4.4%), 285.08 (1.8%), 284.07(1.1%).

5.2.5. 3-N-(2-Methylphenyl)-4,5-tetramethylene thieno[2,3-d] [1,2,3]-triazin-4-(3H)-ones:(*Va*)

M.P: 121–122 °C yield: 51% IR max cm^{-1} = Ar-CH 3044.93: -Ali-CH 2967.98; Arom C=C 1502.37; –CO –1710; (C–N) –739.25. NMR: Solvent –$CDCl_3$ 7.1(t, 3H, Arom)-6.7(s, 1H, Arom), 2.3 (s, 3H, methyl protons), 2.6 (m, 4H, methylenic proton), 1.7(m, 4H, methylenic protons). MS (%) 297.09 (M^+ 100.0%), 298.07 (17.3%), 299.08 (4.5%), 299.10 (1.4%), 298.091(1.1%).

5.2.6. 3-N-(3-methylphenyl)-4,5- tetramethylene thieno[2,3-d] [1,2,3]-triazin-4-(3H)-ones:(*VIa*)

M.P: 138–140 °C yield: 64% IR max cm^{-1} = Ar-CH 3013.34: -Ali-CH 2963.25; Arom C=C 1507.26; –CO –1684; (C–N) –742.25. NMR: Solvent – $CDCl_3$ 7.4(d, 2H, Arom), 7.1(s,1H, Arom), 6.7(s,1H, Arom) 2.4(s,3H, methyl protons), 1.7 (m, 4H, methylenic protons), 2.2 (m,4H, methylenic protons MS (%) 297.80 (M^+ 100.0%), 298.69 (17.3%), 299.88 (4.5%), 299.10 (1.4%), 298.29 (1.1%).

5.2.7. 3-N-(4-methylphenyl)-4,5-tetramethylene thieno[2,3-d] [1,2,3]-triazin-4-(3H)-ones:(*VIIa*)

M.P: 175–176 °C yield: 71% IR max cm^{-1} = -Ar-CH 3012.21: -Ali-CH 2989.11; Arom C=C 1502.37; –CO –1697; (C–N) –762.31. NMR: Solvent – $CDCl_3$ 7.2(d, 2H, Arom)–7.5 (d,2H, Arom), 2.3 (s,3H, methyl protons), 2.8 (m,4H, methylenic proton), 1.5 (m,4H,

methylenic proton). MS (%)297.80 (M^+ 100.0%), 297.14 (17.3%), 298.31 (4.5%), 299.62 (1.4%), 296.02 (1.1%).

*5.2.8. 3-N-(4-flouorophenyl)–4,5-tetramethylene thieno[2,3-d] [1,2,3]- triazin-4-(3H)-ones:(**VIIIa**)*

M.P: 154-156 °C yield: 67%. IR max cm^{-1} = -Ar-CH 3015.61: -Ali-CH 2981.93; Arom C=C 1510.43; –CO –1694; (C–N)–777.26. NMR: Solvent– $CDCl_3$ 7.1 (d, 2H, Arom), 7.8 (d,2H, Arom), 2.6 (m,4H, methylenic proton) 1.5 (m,4H, methylenic protons). MS (%) 301.07 (M^+ 100.0%), 302.07 (18.6%), 303.06 (5.2%), 303.09 (1.7%),

*5.2.9. 3-N-(2-chlorophenyl)–4,5- tetramethylene thieno[2,3-d] [1,2,3]-triazin-4-(3H)-ones: (**IXa**)*

M.P: 145–146 °C yield: 76% IR max cm^{-1} = -Ar-CH 3098.35: -Ali-CH 2968.57; Arom C=C 1508.24; –CO –1688; (C–N) –773.42 NMR: Solvent –$CDCl_3$ 7.9 (s,1H, Arom), 7.2(d,2H, Arom), 6.8 (s,1H, Arom), 2.5 (m,4H, methylenic protons), 1.6 (m,4H, methylenic protons). MS (%) 317.04 (M^+ 100.0%), 319.04(32.5%), 318.04 (18.6%), 320.04 (6.4%), 319.03(4.4%), 321.03(1.4%), 319.05(1.3%).

*5.2.10. 3-N-(3-Chloro phenyl)–4,5-tetramethylene thieno[2,3-d] [1,2,3]-triazin-4-(3H)-ones: (**Xa**)*

M.P: 158–160 °C yield: 71% IR max cm^{-1} = -Ar-CH 3088.35: -Ali-CH 2978.57; Arom C=C 1508.24; –CO –1688; (C–N) –773.42 NMR: Solvent –$CDCl_3$ 7.3(d,2H, Arom), 7.5 (s,1H, Arom), 7.8 (s,1H, Arom), 2.6 (m,4H, methylenic protons), 1.5 (m,4H, methylenic protons). MS (%) 317.06 (M^+ 100.0%), 319.05(33.1%), 318.06 (17.2%), 320.08 (6.9%), 319.03(4.1%), 321.05(1.7%), 319.03(1.2%).

*5.2.11. 3-N-(4-chlorophenyl)-4,5-tetramethylene thieno[2,3-d] [1,2,3]-triazin-4-(3H)-ones: (**XIa**)*

M.P: 110–112 °C yield: 62% IR max cm^{-1} = Ar-CH 3098.35: -Ali-CH 2968.57; Arom C=C 1508.24; –CO –1688; (C–N) –773.42 NMR: Solvent –$CDCl_3$ 7.2(d,2H, Arom), 7.8 (d,2H, Arom), 2.8 (m,4H, methylenic protons),1.6 (m,8H, methylenic protons). MS (%) 317.04 (M^+ 100.0%), 319.04(32.5%), 318.04 (18.6%), 320.04 (6.4%), 319.03 (4.4%), 321.03(1.4%), 319.05(1.3%).

*5.2.12. 3-N-(3-Chloro-4-fluorophenyl)–4,5-tetramethylene thieno [2,3-d][1,2,3]-triazin-4-(3H)-ones: (**XIIa**)*

M.P: 125–126 °C yield: 79% IR max cm^{-1} = Ar-CH 3098.35: -Ali-CH 2968.57; Arom C=C 1508.24; –CO –1688; (C–N) –773.42 NMR: Solvent –$CDCl_3$ 7.7(s,1H, Arom), 7.4(d,2H, Arom), 2.4 and 1.5 (m,8H, tetramethylenic protons). MS (%) 335.03 (M^+ 100.0%), 337.04(37%), 336.03 (18.2%), 338.05 (6.4%), 319.03(4.4%), 321.03 (1.4%), 319.05(1.3%).

Acknowledgement

The authors are thankful to Peoples Education Society management for providing all necessary facilities to carry out the research work.

References

[1] A.M. Khalil, M.A. Berghot, M.A. Gouda, Eur. J. Med. Chem. 44 (11) (2009) 4434–4440.
[2] A. Foroumadi, M. Oboudiat, S. Emami, A. Karimollah, L. Saghaee, M.H. Moshafi, A. Shafiee, Bioorg. Med. Chem. 14 (10) (2006) 3421–3427 15.
[3] C.K. Ryu, S.K. Lee, J.Y. Han, O.J. Jung, J.Y. Lee, S.H. Jeong, Bioorg. Med. Chem. Lett. 15 (10) (2005) 2617–2620.
[4] M. Wujec, M. Pitucha, M. Dobosz, U. Kosikowska, A. Malm, Acta Pharm. 54 (2004) 251–260.
[5] Kumara, H. Tholappanavara, Suresha, Mahadevan, Kittappa, Harishkumar, et al., Phosphorus, Sulfur, Silicon Relat. Elem. 184 (7) (2009) 1866–1879.
[6] M.A. Metwally, F. Bakr, A. Wahab, M. Koketsu, Phosphorus, Sulfur, Silicon Relat. Elem. 184 (11) (2009) 3038–3074 1563–5325.
[7] D.C. Young, T.R. Bailey, PCT. Int. Appl. Wo-2000018231A1 (2000). Chem. Abstr. 132 (2000) 260668.
[8] J.M. Quintela, C. Peinador, L. Gonzalez, R. Iglesias, A. Parama, F. Alvarez, M. L. Sanmartín, R. Riguera, Eur. J. Med. Chem. 38 (3) (2003) 265–275.
[9] M. Brenda, Cleramunt, M. Rosa, Elguero, Josh, Atienza, Juan, Gomez, Barrio, Alicia, Escario, A. Jose, Chem. Pharm. Bull. 39 (8) (1991) 1990–1993.
[10] G. Carmen, G. Fancisco, V. Montserrat, V. Pera, Synthesis 11 (1983) 932–933.
[11] J.N. Sain, Baruah, J.S. Sandu, J. Hetero. Chem. 21 (1984) 257.
[12] B.B. Thomas, F.G.S. Malcolm, J. Chem. Soc. Perkin Trans-I (1975) 1023.
[13] G. Franco, L. Maria, P. Giuseppe, J. Het. Chem. (1989) 613.
[14] D. Gaetano, C. Girolamo, A.M. Anna, A. Enrico, J. Het. Chem. 26 (1989) 1747.
[15] Wortington, P. Anthony, Clough, M. John, Godtrey, R. Christopher, A. Y. Streeting, T. Ian, Chem. Abstr. 118 (1993) 47584g.
[16] J. Saravanan, S. Mohan, Ind. J. Hetero. Chem. 13 (4) (2004) 389–390.
[17] J. Saravanan, S. Mohan, Ind. J. Hetero. Chem. 13 (2003) 185–186.
[18] G.R. Jani, K.B. Vyas, Z. Franco, E-J. Chem. 6 (4) (2009) 1228–1232.
[19] C. Jim, H. George, J.Chem.Soc. Perkin. Trans. I (2005) 1984.
[20] K.M. Khan, Z. Nullah, M.A. Lodhi, S. Jalil, M.I. Choudhary, A.U. Rahman, J. Enz Inhib. Med. Chem. 21 (2) (2006) 139–143.
[21] K. Gewald, E. Schinke, H. Bohcher, Chem. Ber. 99 (1966) 94–100.
[22] A.L. Barry, Antimicrobic susceptibility test: principle practices. Biol. Abstr. 64 (5) (1977) 25183.
[23] C.H. Collins, Microbiological Methods. Butter worths Ed. II, London, 1967.