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O-methyl 4-((substituted)amino)-5-methylpyrrolo[2,1-*f*][1,2,4]triazine-6-carbothioate: Synthesis and Pharmacological Studies

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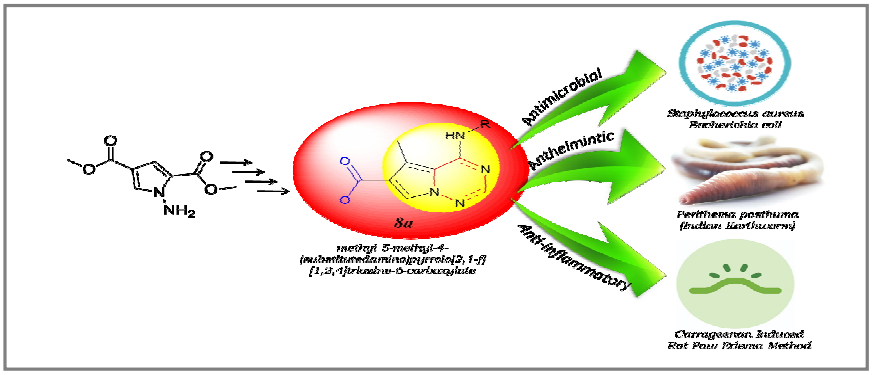
ABSTRACT

*A novel series of O-methyl 4-((substituted)amino)-5-methylpyrrolo[2,1-f][1,2,4]triazine-6-carbothioate derivatives (**8a-j**) were synthesized by the reaction of O-methyl 5-methyl-4-(methylthio) pyrrolo[2,1-f][1,2,4]triazine-6-carbothioate **(6)** with different substituted aromatic and aliphatic amines. All the compounds were characterized using Liquid Chromatographic Mass Spectra, Infra Red, Proton and Carbon Nuclear Magnetic Resonance spectral data's and synthesized derivatives were screened for antimicrobial, anthelmintic and anti-inflammatory activities. Out of all the test samples tested for biological potency, compounds **8a, 8b, 8e, 8f, 8g** and **8j** showed significant potency.*

Graphical Abstract



Keywords: 1,2,4-Triazine, *Perithema posthuma*, *Carrageenan*, Intramolecular cyclization

INTRODUCTION

In Nature nitrogen containing heterocycles are very found abundantly in both plants and animals. The countless biological potency was drive from these heterocycles ranging from serotonin/melatonin (neurological secondary messengers) to facilitate the crucial hydrogen-bonding frame that makes the base of life with macromolecules like DNA and RNA. As a result, it is not surprising that similarities of natural condensation of nitrogen heterocycles observed in purines and indoles have found usefulness in pharmaceutical and agrichemical industrial applications. Owing to this, special interests are on heterocycles having N-N bond with one of the two nitrogen atoms placed at the bridgehead point of the bicyclic ring system. One of 3 isomers of the 6 membered ring structure having three nitrogen atoms is 1,2,4-triazine. In the late 70s a distinctive N-N bond Pyrrolo[2,1-*f*][1,2,4]triazine, with a nitrogen bridge was first synthesized [**1**] of the very small number of alternatives that fall inside this scope of fused heterocycles, one heterocycle is pyrrolo[2,1-*f*][1,2,4]triazine. It has delivered a stage from which a different and noteworthy pharmaceutical work has advanced. Pyrrolo[2,1-*f*][1,2,4]triazine lingered at the outer reaches of medicinal chemistry till a convergence of researchers, urged by the outburst of the kinase inhibitor research field and demanding requirement for new, potent drug molecules to fit in that stimulating research field with varied therapeutic objectives.[**2, 3**].

Literature review have proved that pyrrolo[2,1-*f*][1,2,4]triazine system can act as a bioisosteric alternatives to quinazoline, pyrimidine, adenine ring systems in addition to advantageous structural frame that has been found in to be proven drug contenders [**4**]. Predominant pharmacological effects observed in molecules with 1,2,4-triazine core moiety are antitumor [**5**], anti-AIDS [**5**], CRF receptor antagonists [**6**], anti-microbial and anti-inflammatory [**7**]. Symbolically most of the known drugs marketed like azaribine [**8**], lamotrigine [**9**], tirapazamine (TPZ)[**10**] used as antiviral, antiepileptic and anticancer encompass 1,2,4-triazine nucleus. Pyrrolo[2,1-*f*][1,2,4]triazine are known to possess numerous biological activities such as antifungal [**11**], anti-inflammatory [**12**], anticancer [**13**], antitubercular [**14**], antibacterial [**15**], antihepatitis [**16**], insecticidal [**17**], anti-HIV[**18**] and antitumor activities[**19**].The sequence of NCNN in 1,2,4-triazine ring structure has been considered to be the essential feature for the molecule to exhibit various pharmacological activities such as Met kinase inhibitor with *in vitro* nanomolar kinase potency with binding in an ATP manner [**20**] , pan-Aurora kinase inhibitors [**21**] , *in vitro* potency against VEGFR-2 kinase [**22, 23**], and *in vivo* IRAK4 inhibition [**24**] properties. Encouraged by the findings of research on biological activities of substituted pyrrolo[2,1-*f*][1,2,4]triazines, attempts was made to study to look for new synthetic route for synthesis of O-methyl 4-((substituted)amino)-5-methylpyrrolo[2,1-*f*][1,2,4]triazine-6-carbothioate derivatives **(8a-j)** as shown in the Scheme and to evaluate the biological properties.

MATERIALS AND METHODS

Synthesis of dimethyl 1*H*-pyrrole-2,4-dicarboxylate (2): A mixture of (*E*)-methyl but-2-enoate **(1)** (0.1 mol, 10 g) and sodium hydride (1.5 eq, 35.95 g) was taken in round bottom flask and initially for 3 min. Toluene sulfonyl methyl isocyanide (TOSMIC) (1eq, 19.5 g) in dry THF (50 mL) was added to the reaction mixture slowly under stirring. Stirring continued for another 1.5 h and completion of the reaction monitored by TLC. Reaction mixture was quenched with saturated solution of ammonium chloride, extracted with ethyl acetate, dried over sodium sulfate and concentrated under reduced pressure to get crude dimethyl 1*H*-pyrrole-2,4-dicarboxylate **(2)**. Crude compound **(2)** was further purified by column chromatography technique using 7:3 Pet ether: Ethyl acetate solvent system.

Synthesis of dimethyl 1-amino-1*H*-pyrrole-2,4-dicarboxylate (3): A mixture of dimethyl 1*H*-pyrrole-2,4-dicarboxylate **(2)**(0.1 mol, 18.3 g),Sodium hydride taken in THF (50 mL), was stirred for 10 min. N-(Diphenylphosphinothioyl)hydroxylamine (1 eq, 24.9 g) was added at once and stirred vigorously. Stirring of reaction mixture was continued overnight at room temperature and TLC method was used to monitored completion of the reaction. Reaction mixture was diluted with ethyl

acetate and extracted, organic layer obtained was washed with water, dried over sodium sulfate and concentrated under reduced pressure to get crudedimethyl 1-amino-1*H*-pyrrole-2,4-dicarboxylate**(3)**. Further, the crude compound was purified by column chromatography using 1:1 Ethyl Acetate: Petroleum Ether over Neutral Alumina to get pure compound **(3)** with good yield and purity.

Synthesis of methyl 5-methyl-4-oxo-3,4-dihydropyrrolo[2,1-*f*][1,2,4]triazine-6-carboxylate (4): Dimethyl 1-amino-1*H*-pyrrole-2,4-dicarboxylate**(3)** (0.1 mol, 19.8 g) inFormamide (10 vol) were taken in 500 mL round bottom flask and refluxed at 220°C for 5 h. Completion of reaction was checked by TLC, excess formamide was removed under pressure to get crude methyl 5-methyl-4-oxo-3,4-dihydropyrrolo[2,1-f][1,2,4]triazine-6-carboxylate **(4)**. The solid crude obtained was purified by trituration in petroleum ether to remove any non-polar impurities followed by increasing the polarity with addition of ethyl acetate to remove any polar impurities.The crude was taken to the next step without purifying further.

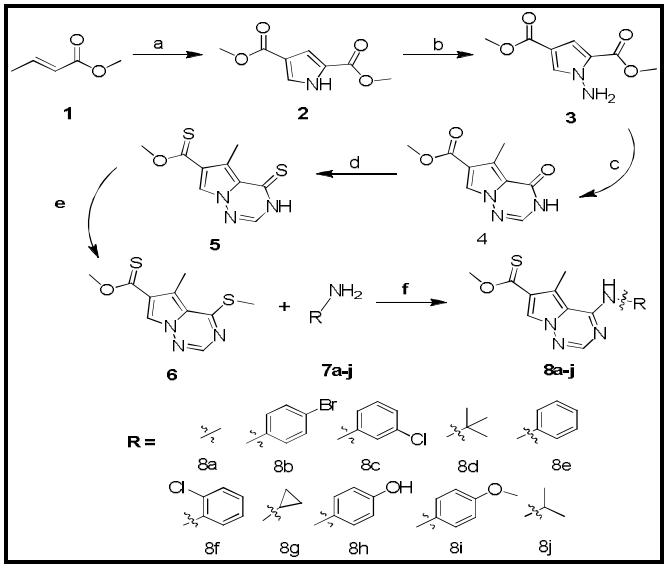
Synthesis of methyl 5-methyl-4-thioxo-3,4-dihydropyrrolo[2,1-*f*][1,2,4]triazine-6-carboxylate(5): Mixture of methyl 5-methyl-4-oxo-3,4-dihydropyrrolo[2,1-*f*][1,2,4]triazine-6-carboxylate**(4)** (0.1 mol, 20.7 g) was taken in Chloroform (250 mL) followed by addition of sulphur powder (5 g) and refluxed overnight at 120°C. Completion of the reaction was confirmed by TLC. Excess of chloroform was removed under reduced pressure, solid obtained was triturated with ethyl acetate and petroleum ether to remove any colour impurities to get 5-methyl-4-thioxo-3,4-dihydropyrrolo[2,1-*f*] [1,2,4]triazine-6-carboxylate **(5)**. The crude was taken to the next step without purifying further.

Synthesis of methyl 5-methyl-4-(methylthio)pyrrolo[2,1-*f*][1,2,4]triazine-6-carboxylate (6): Mixture of 5-methyl-4-thioxo-3,4-dihydropyrrolo[2,1-*f*][1,2,4]triazine-6-carboxylate **(5)** (0.1 mol, 22.3 g) was taken in dry THF with addition of methyl iodide (0.1 mol, 14.1 g)very slowly and the reaction mixture was heated to 80°C for 4 h. TLC was used to monitor the completion of the reaction, excess of THF was distilled off, the crude methyl 5-methyl-4-(methylthio)pyrrolo[2,1-*f*][1,2,4] triazine-6-carboxylate **(6)** obtained as solid was used in the next step without any further purification.

General procedure for the preparation of O-methyl 4-((substituted)amino)-5-methylpyrrolo[2,1-*f*][1,2,4]triazine-6-carbothioate (8a-j): Equimolar mixture of methyl 5-methyl-4-(methylthio) pyrrole[2,1-*f*][1,2,4]triazine-6-carboxylate **(6)** (0.001 mol, 0.23 g), different substituted aliphatic and aromatic amines**(7a-j)** were taken in the three necked round bottom flask. The resulting reaction mixture was refluxed at 90°C for 6h. After completion of reaction, organic phase washed thoroughly with water, dried over magnesium sulphate followed by recovery of solvent under pressure. Residue attained was purified by column chromatography with (1:1) $CHCl_3$/MeOH as eluent to get pure novel series O-methyl 4-((substituted)amino)-5-methylpyrrolo[2,1-*f*] [1,2,4] triazine-6-carbothioate derivatives **(8a-j)** in good yield. Physical data of synthesised title compounds were given in table 1.

Reagents and conditions: a) NaH, TOSMIC/THF, 0 - r.t, 2 h; b) NaH, $Ph_2P(O)ONH_2$/ THF, r.t, 12 h; c) NH_2CHO, 220°C, 5 h; d) S/$CHCl_3$, 120°C, 12 h; e) MeI/THF, 80°C, 4 h; f) Substituted amines (7a-j), 0 to 90°C, 6 h.

Antimicrobial Activity: Bacterial strains (*Staphylococcus aureus* and *Escherichia coli*) were purchased form CSIR-National Chemical Laboratory (NCL) Pune. Agar well diffusion technique was made use for the antibacterial study by means of standard drug Ciprofloxacin as standard drug, obtained from Himedia, Mumbai. *Staphylococcus aureus* a Gram +ve (NCIM-5022) and *Escherichia coli* (NCIM-5051) a Gram -ve bacterial strains were used for the study *in vitro* antibacterial potency. *Candida albicans* (ATCC-10231) and *Microsporum gypseum* (ATCC-10215) stock fungal moulds procured from Institute of Microbial Technology (IMTECH), Chandigarh, India was used for the study. *In vitro* antifungal assay using Poisoned Food Technique of all the synthesized O-methyl 4-((substituted)amino)-5-methylpyrrolo[2,1-*f*][1,2,4]triazine-6-carbothioate derivatives **(8a-j)** was tested out taking Standard drug Ketoconazole was acquired from Himedia, Mumbai.



Scheme 1. Preparation of O-methyl 4-((substituted)amino)-5-methylpyrrolo [2,1-*f*][1,2,4]triazine-6-carbothioate (8a-j)

Antibacterial Activity: To study antibacterial nature of the synthesized molecules by broth culture under the sterilized condition "Agar well diffusion technique" [25-27] was made use. A small sample of bacterial culture was removed from working culture into 15-20 mL sterile 0.9% Normal Saline solution (NaCl solution) with gentle mixing. Warm agar media and approximately 0.75 mL of inoculums was transferred into decontaminated petri dish, allowed to solidify and incubated for 24 h. On neutralized nutrient agar medium with the aid of sterilized L-Shaped glass rod test bacterial strains were evenly smeared on to the agar media. Five wells of 7 mm size were punched in to the media with the help of cork borer to billet 50 µL of the test sample solutions in dimethyl sulfoxide with varied concentrations 5, 15, 25, 35 and 45 µg µL^{-1} and negative control dimethylsulfoxide (DMSO). Micro dilution technique was made use to identify the Minimum Inhibitory Concentration (MIC) values for all the series compounds of concentrations and are represented in figure 1. Ciprofloxacin (20 µg µL^{-1}) a standard drug selected as positive control was procured from Himedia, Mumbai. All the test samples **(8a-j)** minimum inhibitory concentrations values was observed to be 45 µg µL^{-1}. After loading of standard, test samples, and control into respective wells by means of new sterilized micropipette tips and were kept for incubation at temperature of 37°C for about 36 h. At the end of incubation time, diameter of zone of inhibition of each well measured in mm with the help of a ruler. To get the concordant values, experiment voted for triplicate study and represented in figure 2.

Antifungal Activity: Antifungal activity of the synthesized molecules were investigated against *Candida albicans* and *Microsporum gypseum* fungi moulds by poisoned food technique "Potato Dextrose Agar (PDA) medium" [28-30] and standard fungicide Ketoconazole. Initial potato soup was made by crushing boiled potato in water, filtered and mixed well with dextrose, agar. The mixture was decontaminated in autoclave at 120°C for 20 min and cooled to just above room temperature (~ 35°C). Approximately 15 mL, sterile molten potato dextrose agar was dispensed into sterilized petri plates and allowed to stand to solidify. Minor portions of the mycelium of two fungus were inoculated cautiously on to potato dextrose agar media plates with L-shaped decontaminated glass rod. After completion of inoculation of petri plates were set for incubation for nearly five days at (24 ± 2)°C. Six even size wells of 6 mm diameter were bored in to inoculated media with the help of sterile cork borer capable of holding 50 µL of the test sample, standard solution, and control. Test sample solutions were made by dissolving in dimethyl sulfoxide to achieve various concentrations 10, 20, 30, 40 and 50 µg µL^{-1} against negative control dimethyl sulfoxide (DMSO). Minimum Inhibitory Concentration (MIC) values found from microdilution technique was found to be 50 µg µL^{-1} and above is reported in figure 3. The test samples **(8a-j)** at concentration 50 µg µL^{-1} per well was used to screen for the

antifungal activity. In order to offer adequate time for the fungi to spread over a substantial area of the petri plates, the plates were then kept for gestation for about 24 h at 4°C and another 74 h incubation at 28°C. Assay was carried out in triplicates and zone of inhibition diameter is measured in mm and the values are depicted in figure 4.

Anthelmintic Activity: Indian Earthworm (*Pheretima posthuma*) were used to carry out the anthelmintic activity for O-methyl 4-((substituted)amino)-5-methylpyrrolo[2,1-*f*][1,2,4]triazine-6-carbothioate derivatives (**8a-j**). Worms were preserved under normal vermin composting environment with bearable supply of nourishment and liquids for three weeks. Piperazine citrate was procured from SD Fine Chemical Ltd., Mumbai and was taken as Standard drug. Sodium chloride also known as normal saline (*NS*) 0.90% w/v was prepared using distilled water for the investigation in the research laboratory. Completely mature earthworms of approximately 6 cm in length and 0.4-0.6 cm in thickness were chosen for the analysis. Treatments of 50 mg of test samples were carried out according to standard methods [**31**]. Petriplates each with six earthworms was taken as one set, earthworms were eroded separately with normal saline and kept in 25 mL of normal saline. First set of earthworms were taken as control and kept in 25 mL saline in sterile petri plate and second set of earthworms were kept in 25 mL saline along with standard drug Piperazine Citrate (50 mg mL^{-1}) and was considered as positive control. Accordingly, all the remaining sets of worms were taken in 25 mL saline along with 50 mg mL^{-1} of test samples. Minimum Concentration of pyrrolo[1,2-*f*][1,2,4] triazines **(8a-j)** were obtained on the basis of full study of anthelmintic activity figure 5 of the test samples at different concentration 10, 20, 30, 40 and 50 mg mL^{-1} show 50 mg mL^{-1} as the minimum concentration value. Test systems were observed for 4 h for signs of paralysis followed by death resulting in loss of motility and body decolouration. Time taken for paralysis and time taken for death were fixed as objective and the same was reported in minutes. Non-revival body posture in the normal saline medium was considered as time of paralysis and loss in total motility with bleached body color was determined as time of death, based on the results represented in figure 6.

Anti-inflammatory Activity: Albino Wistar rats both male and female weighing between 145-175 g were acquired from Sree Venkateshwara Enterprises Bangalore and accustomed by keeping for about 1 week at normal experimental environment, given free access to UV purified water and with pelleted feed supplied purchased from M/s Pranava Agro Industries, Sangli, Maharastra. All analysis shown were as genuine by the Institutional Animal Ethics Committee (IAEC) of Sree Siddaganga College of Pharmacy, Tumkur (Ref No: SSCPT/IAEC.Clear/141/2012-13),standard Indomethacin, procured from Lab Fine Chem Industries. Test samples of conentrtions100 mg kg^{-1} body weight and standard of 10 mg kg^{-1} body weight was taken for the study. Vehicle Carboxy Methyl Cellulose 1% well known as CMC was chosen for oral route administration for the test animals. Carrageenan induced rat paw edema method [**32, 33**] was executed on test animals, they were randomly divided into three sets with 6 test animals in each individual groups off our ten sets separately for screening pyrrolo[1,2-*f*][1,2,4] triazine **(8a-j)** derivatives and standard drug. Test animals were famished overnight in advance start of test, first group assisted as control i.e., vehicle Carrageenan Inducer, second group assisted as standard having Indomethacin given through orally. Third to fourteenth groups are all the series of synthesized compounds were administered at concentration 100 mg kg^{-1} b w. After administration, test animals were observed for 0 to 30 min for some clinical indications. Instantly after inoculation sub plantar section of the right back paw, immediately paw size was recorded using Plethysmometer, same recording procedure was repetitive at 30 min, 60 min, 120 min and 180-min time inter missions to record paw size and the readings are represented in figure 7.

RESULTS AND DISCUSSION

Chemistry: Synthesis of O-methyl 4-((substituted)amino)-5-methylpyrrolo[2,1-*f*][1,2,4]triazine-6-carbothioate derivatives **(8a-j)** is as shown in the scheme 1. Initial reaction of (*E*)-methyl but-2-enoate **(1)** with sodium hydrideand Toluenesulfonyl methyl isocyanide (TOSMIC) in THF gave dimethyl 1*H*-pyrrole-2,4-dicarboxylate **(2)** as solid. Compound **(2)** was treated with sodium hydride

and N-(Diphenylphosphinothioyl) hydroxylamine in THF to get dimethyl 1-amino-1*H*-pyrrole-2,4-dicarboxylate**(3)**. Dimethyl 1-amino-1*H*-pyrrole-2,4-dicarboxylate **(3)** in Formamide and refluxed at 220°C for 5 h to achieve methyl 5-methyl-4-oxo-3,4-dihydropyrrolo[2,1-*f*][1,2,4]triazine-6-carboxylate **(4).** Mixture of compound **(4)** taken in excess of Chloroform followed by addition of sulphur powder and refluxed overnight at 120°C yielded in methyl 5-methyl-4-thioxo-3,4-dihydropyrrolo[2,1-*f*][1,2,4]triazine-6-carboxylate **(5).** This tailed by slow treatment of 5-methyl-4-thioxo-3,4-dihydropyrrolo[2,1-*f*][1,2,4]triazine-6-carboxylate **(5)** with methyl iodide in dry THF at 80°C for 4 h resulted in methyl 5-methyl-4-(methylthio)pyrrolo[2,1-*f*][1,2,4]triazine-6-carboxylate **(6)**. This pre final compound **(6)** upon treatment with different aliphatic and aromatic amines **(7a-j)** at 90°C for 6 hours resulted in novel *O*-methyl substituted pyrrolo[2,1-*f*][1,2,4]triazine-6-carbothioates **(8a-j)** in good yield, Physical data of synthesised title compounds were given in table 1.

Table 1. Physical Data of O-methyl 4-((substituted)amino)-5-methylpyrrolo [2,1-*f*][1,2,4]triazine-6-carbothioate derivatives **(8a-j)**

Comp. Code	Molecular Formula	Molecular Weight	Melting Point (mp°C)	Yield (%)
8a	$C_{10}H_{12}N_4OS$	236.29	243-245	75
8b	$C_{15}H_{13}BrN_4OS$	377.25	244-246	69
8c	$C_{15}H_{13}ClN_4OS$	332.80	292-294	86
8d	$C_{13}H_{18}N_4OS$	278.37	289-291	70
8e	$C_{15}H_{14}N_4OS$	298.36	286-289	78
8f	$C_{15}H_{13}ClN_4OS$	332.80	235-237	90
8g	$C_{12}H_{14}N_4OS$	262.33	265-267	80
8h	$C_{15}H_{13}ClN_4OS$	332.80	262-264	68
8i	$C_{16}H_{16}N_4O_2S$	328.38	115-117	76
8j	$C_{12}H_{16}N_4OS$	264.34	221-223	85

Spectral Interpretation of Synthesized Compounds:

O-methyl 5-methyl 4-(methylamino)pyrrolo[2,1-*f*][1,2,4]triazine-6-carbothioate (8a): IR (KBr, cm^{-1}): 2926.45 (-CH_3 sp3 Stretching), 1226.5 (C-N stretching), 1240.97 (C-O Ether bond stretching), 3136.65 (N-H Stretching) and 758.85 (N-H Out of Plane Stretching)**;** ^{1}**H NMR** (399.6 MHz, $CDCl_3$) δ ppm 8.07 (s, 1H, Ar-H), 8.02 (s, 1H, Ar-H), 3.88 (s, 3H, OCH_3), 2.83 (s, 3H, NH-CH_3), 2.64 (s, 3H, CH_3), 1.61 (s, 1H, NH); ^{13}**CNMR** (100 MHz, $CDCl_3$) δ ppm 168.06, 164.86, 146.48, 121.31, 120.74, 118.18, 116.17, 51.41, 12.12, 12.10**; LC-MS** m/z Calculated (Found) 236.29 (237.40).

O-methyl4-(cyclopropylamino)-5-methylpyrrolo[2,1-*f*][1,2,4]triazine-6-carbothioate(8b): ^{1}**HNMR** (399.6 MHz, $CDCl_3$) δ ppm 8.06 (s, 1H, Ar-H), 8.025 (s, 1H, Ar-H), 7.35 (d, 2H, Ar-H), 7.01 (d, 2H, Ar-H), 3.91 (s, 1H, NH), 3.88 (s, 3H, OCH_3), 2.83 (s, 3H, NH-CH_3); ^{13}**C NMR** (100 MHz, $CDCl_3$) δ ppm 205.01, 149.30, 147.0, 139.9, 132.40, 132.40, 127.80, 124.40, 119.50, 118.50, 118.50, 116.70, 116.5, 53.60, 10.20; **LC-MS** m/z: Calculated 377.26.

O-methyl 4-(isopropylamino)-5-methylpyrrolo[2,1-*f*][1,2,4]triazine-6-carbothioate (8c): ^{1}**H NMR** (399.6 MHz, $CDCl_3$) δ ppm 8.06 (s, 1H, Ar-H), 8.025 (s, 1H, Ar-H), 7.51 (d, 1H, Ar-H), 7.14 (t, 1H, Ar-H), 6.85 (t, 1H, Ar-H), 3.91 (s, 1H, NH), 3.88 (s, 3H, OCH_3), 2.83 (s, 3H, NH-CH_3); ^{13}**CNMR** (100 MHz, $CDCl_3$) δ ppm 205.01, 149.30, 147.0, 142.70, 135.10, 130.90, 124.40, 122.3, 119.5, 116.7, 116.5, 115.9, 53.60, 10.20; **LC-MS** m/z: Calculated 332.81.

O-methyl 4-(tert-butylamino)-5-methylpyrrolo[2,1-*f*][1,2,4]triazine-6-carbothioate (8d): IR(KBr, cm^{-1}): 2926.45 (-CH_3 sp3 Stretching), 1226.5 (C-N stretching), 1240.97 (C-O Ether bond stretching),3136.65(N-H Stretching) and 758.85 (N-H Out of Plane Stretching)**;** ^{1}**H NMR** (399.6 MHz, $CDCl_3$) δ ppm 8.07 (s, 1H, Ar-H), 8.23 (s, 1H, Ar-H),3.87 (s, 3H, OCH_3), 2.63 (s, 3H, NH-CH_3),1.61 (s, 1H, NH), 1.20 (s, 9H, (-CH_3)$_3$)**;** ^{13}**C NMR** (100 MHz, $CDCl_3$) δ ppm 168.06, 164.86, 146.48, 121.31, 120.74, 118.18, 116.17, 51.41, 12.12, 12.10, 4.86**; LC-MS** m/z Calculated (Found) 278.37 (279.17).

O-methyl 5-methyl-4-(phenylamino)pyrrolo[2,1-*f*][1,2,4]triazine-6-carbothioate (8e): **^{1}HNMR** (399.6 MHz, $CDCl_3$) δ ppm 8.06 (s, 1H, Ar-H), 8.02 (s, 1H, Ar-H), 7.63 (d, 2H, Ar-H), 7.20 (t, 2H, Ar-H), 6.81 (t, 1H, Ar-H), 3.97 (s, 1H, NH), 3.88 (s, 3H, OCH_3), 2.83 (s, 3H, NH-CH_3); **^{13}CNMR** (100 MHz, $CDCl_3$) δ ppm 205.01, 149.30, 147.0, 129.50, 129.50, 127.80, 127.20, 124.40, 122.40, 119.5, 117.80, 117.80, 116.5, 53.60, 10.20; **LC-MS** m/z: Calculated 332.81.

O-methyl 4-((2-chlorophenyl)amino)-5-methylpyrrolo[2,1-*f*][1,2,4]triazine-6-carbothioate (8f): **^{1}H NMR** (399.6 MHz, $CDCl_3$) δ ppm 8.06 (s, 1H, Ar-H), 8.025 (s, 1H, Ar-H), 7.51 (d, 1H, Ar-H), 7.14 (t, 1H, Ar-H), 6.85 (t, 1H, Ar-H), 3.91 (s, 1H, NH), 3.88 (s, 3H, OCH_3), 2.83 (s, 3H, NH-CH_3); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm 205.01, 149.30, 147.0, 142.70, 135.10, 130.90, 124.40, 122.3, 119.5, 116.7, 116.5, 115.9, 53.60, 10.20; **LC-MS** m/z: Calculated 332.80.

O-methyl 4-((3-chlorophenyl)amino)-5-methylpyrrolo[2,1-*f*][1,2,4]triazine-6-carbothioate (8g): **IR** (KBr, cm^{-1}): 2947.6 (-CH_3 sp3 stretching), 1233.2 (C-N stretching), 1277.6 (C-O Ether bond stretching),3105.8 (N-H Stretching) and 762.7 (N-H Out of Plane Stretching)**;** **^{1}H NMR** (399.6 MHz, $CDCl_3$) δ ppm 8.07 (s, 1H, Ar-H), 8.025 (s, 1H, Ar-H),3.87 (s, 3H, OCH_3), 2.63 (s, 3H, NH-CH_3), 2.27-2.17 (s, 2H, NH and -CH), 1.30 (d, 4H, (-CH_2), 1.11 (d, 4H, (-CH_2)**;** **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm 168.06, 164.86, 146.48, 121.31, 120.74, 118.18, 116.17, 51.41, 12.12, 12.10, 8.18; **LC-MS** m/z Calculated (Found) 262.33 (263.03)**.**

O-methyl 4-((4-chlorophenyl)amino)-5-methylpyrrolo[2,1-*f*][1,2,4]triazine-6-carbothioate (8h): **^{1}H NMR** (399.6 MHz, $CDCl_3$) δ ppm 8.06 (s, 1H, Ar-H), 8.025 (s, 1H, Ar-H), 7.66 (d, 2H, Ar-H), 7.24 (t, 2H, Ar-H), 3.91 (s, 1H, NH), 3.88 (s, 3H, OCH_3), 2.83 (s, 3H, NH-CH_3); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm 205.01, 149.30, 147.0, 139.00, 129.60, 129.60, 127.80, 127.70, 124.40, 121.10, 121.10, 119.5, 116.5, 53.60, 10.20; **LC-MS** m/z: Calculated 332.81.

O-methyl 4-((4-methoxyphenyl)amino)-5-methylpyrrolo[2,1-*f*][1,2,4]triazine-6-carbothioate (8i): **^{1}H NMR** (399.6 MHz, $CDCl_3$) δ ppm 8.06 (s, 1H, Ar-H), 8.025 (s, 1H, Ar-H), 7.51 (d, 1H, Ar-H), 7.14 (t, 1H, Ar-H), 6.85 (t, 1H, Ar-H), 3.99 (s, 1H, NH), 3.90 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 2.83 (s, 3H, NH-CH_3); **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm 205.01, 153.30, 149.30, 147.0, 142.70, 133.20, 127.80, 124.40, 121.70, 121.70, 119.50, 116.70, 115.10, 115.10, 55.80, 53.60, 10.20; **LC-MS** m/z: Calculated 332.81.

O-methyl 4-((4-bromophenyl)amino)-5-methylpyrrolo[2,1-*f*][1,2,4]triazine-6-carbothioate (8j): **IR** (KBr, cm^{-1}): 2926.45 (-CH_3 sp3 Stretching), 1226.5 (C-N stretching), 1240.97 (C-O Ether bond stretching), 3136.65 (N-H Stretching) and 758.85 (N-H Out of Plane Stretching)**;** **^{1}H NMR** (399.6 MHz, $CDCl_3$) δ ppm 8.07 (s, 1H, Ar-H), 8.025 (s, 1H, Ar-H), 3.87 (s, 3H, OCH_3), 3.27-3.13 (m, 2H, NH and -CH), 2.63 (s, 3H, NH-CH_3),1.38-1.36 (d, 6H, (-CH_3)$_2$)**;** **^{13}C NMR** (100 MHz, $CDCl_3$) δ ppm 168.06, 164.86, 146.48, 121.31, 120.74, 118.18, 116.17, 58.41, 51.41, 28.31, 12.12, 12.10; **LC-MS** m/z Calculated (Found) 264.35 (265.30)**.**

Micro dilution technique was made use to identify the Minimum Inhibitory Concentration (MIC) values for all the test samples **(8a-j)** at different concentration 5, 15, 25, 35 and 45 µg mL^{-1} minimum inhibitory concentrations values was observed to be 45 µg mL^{-1}.

All the test compounds O-methyl 4-((substituted)amino)-5-methylpyrrolo[2,1-*f*][1,2,4]triazine-6-carbothioate derivatives **(8a-j)** showed moderate to good inhibitory action against two tested strains *Escherichia coli* and *Staphylococcus aureus*. At first different concentrations of 5, 15, 25, 35 and 45 µg μL^{-1} was carried out against the two bacterial strains. At and above 45 µg μL^{-1} concentration, Minimum Inhibitory Concentration (MIC) was observed reported in figure 1. Compounds **8a**, **8d**, **8e**, and **8i** bared significant antibacterial activity and remaining all compounds showed moderate activity against both *Escherichia coli* and *Staphylococcus aureus* data presented in figure 2.

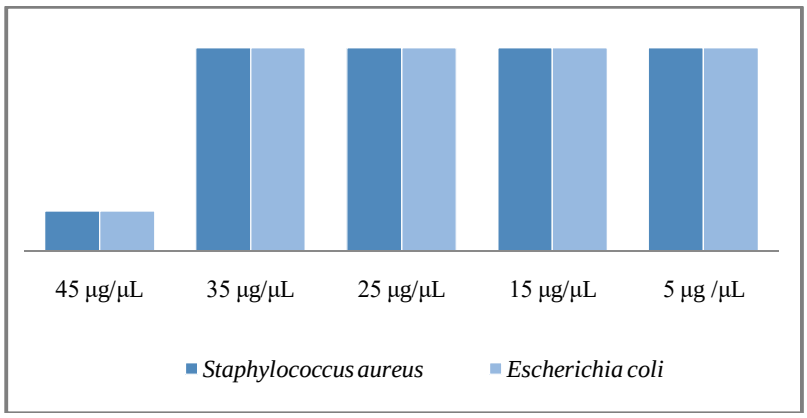


Figure 1. Antibacterial activity; Minimum Inhibitory Concentration (MIC) values of O-methyl 4-((substituted)amino)-5-methylpyrrolo[2,1-*f*][1,2,4]triazine-6-carbothioate derivatives **(8a-j).**

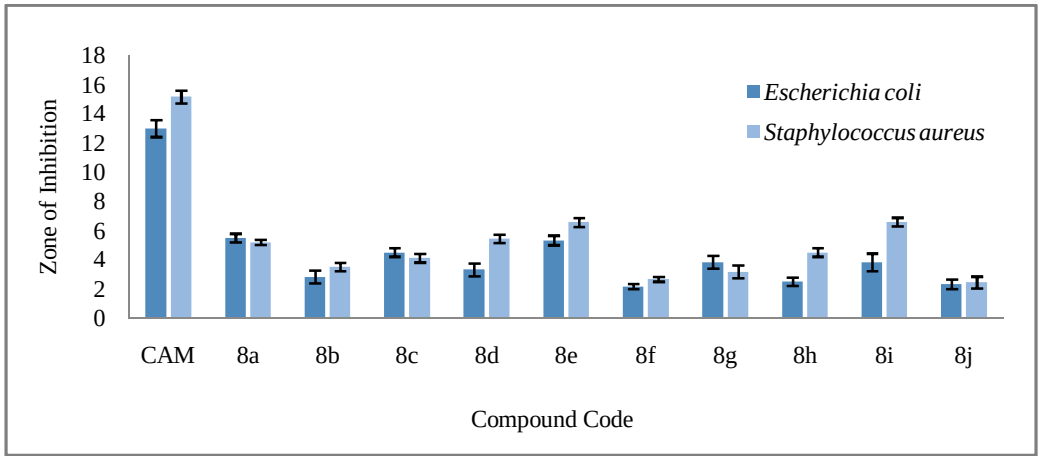


Figure 2. Antibacterial activity Zone of Inhibition values of O-methyl 4-((substituted)amino)-5-methylpyrrolo[2,1-*f*][1,2,4]triazine-6-carbothioate derivatives **(8a-j).**

Micro dilution technique was made use to identify the Minimum Inhibitory Concentration (MIC) values for all the test samples **(8a-j)** at different concentration 10, 20, 30, 40 and 50 µg mL^{-1} minimum inhibitory concentrations values was observed to be 50 µg mL^{-1}.

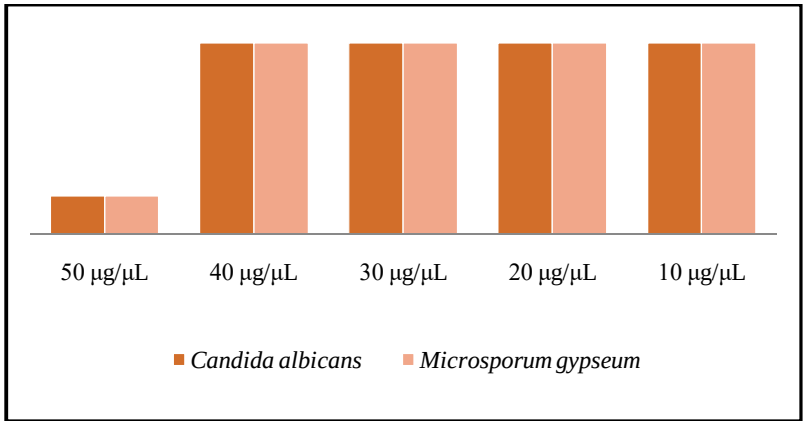


Figure 3. Antifungal activity; Minimum Inhibitory Concentration (MIC) values of O-methyl 4-((substituted)amino)-5-methylpyrrolo[2,1-*f*][1,2,4]triazine-6-carbothioate derivatives **(8a-j).**

All the test compounds O-methyl 4-((substituted)amino)-5-methylpyrrolo[2,1-*f*][1,2,4]triazine-6-carbothioate derivatives **(8a-j)** showed moderate to good inhibitory action against two tested two fungal moulds. At first different concentrations of 10, 20, 30, 40 and 50 µg µL^{-1} was carried out against the two fungal molds. At and above 50 µg µL^{-1} concentration, Minimum Inhibitory Concentration (MIC) was observed test compounds figure 3. All compounds showed moderate to good activity against *Candida albicans* and *Microsporumgypseum*, data presented in figure 4.

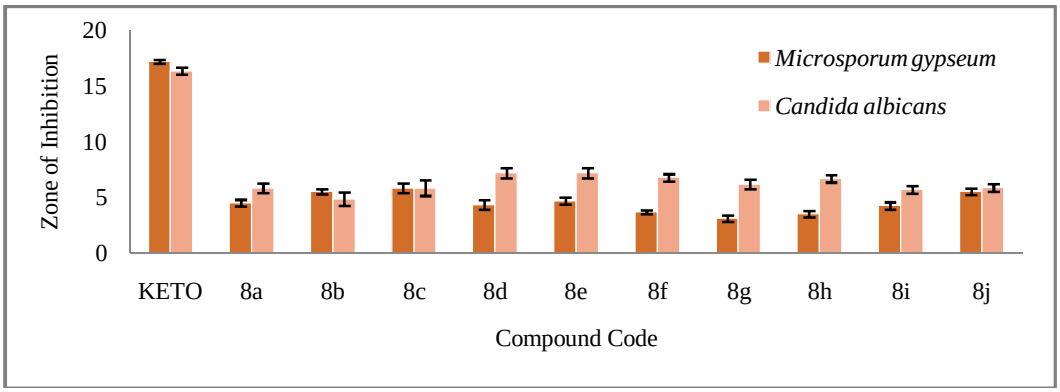


Figure 4. Antifungal activity Zone of Inhibition values of O-methyl 4-((substituted)amino)-5-methylpyrrolo [2,1-*f*][1,2,4]triazine-6-carbothioate derivatives **(8a-j)**.

Minimum Concentration of pyrrolo[1,2-*f*][1,2,4]triazines **(8a-j)** were obtained on the basis of full study of anthelmintic activity of the test samples at different concentration 10, 20, 30, 40 and 50 mg mL^{-1} show 50 mg mL^{-1} as the minimum concentration value.

Anthelmintic property against *Pheretima posthuma* (Indian Earthworm) exhibited moderate anthelmintic effects compared to standard drug Piperazine Citrate. Control set displayed paralysis time at 141.30±0.49 min and death time at 165.17±0.12 min at 50 mg mL^{-1} which the minimum concentration is observed for activity figure 5. Standard drug piperazine citrate 50 mg mL^{-1} exhibited paralysis time at 39.17±0.48 min and death time at 57.67±0.88 min. O-methyl 4-((substituted)amino)-5-methylpyrrolo[2,1-*f*][1,2,4]triazine-6-carbothioate derivatives **(8a-j)** presented good anthelmintic potency at concentration of 50 mg mL^{-1} as reported in figure 6.

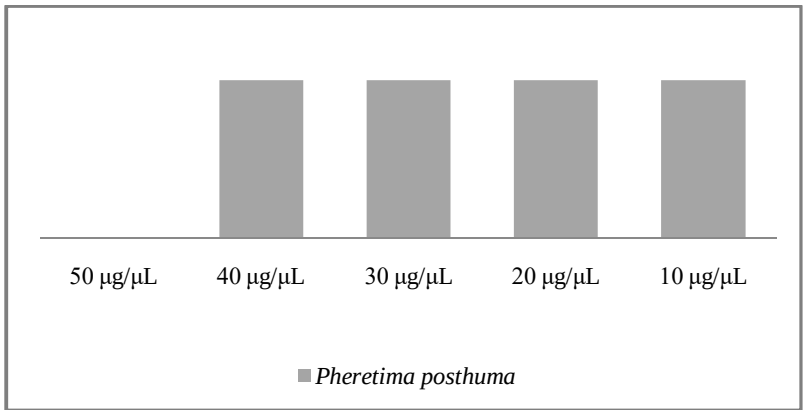


Figure 5. Anthelmintic activity Minimum Inhibitory Concentration (MIC) values of O-methyl 4-((substituted)amino)-5-methylpyrrolo[2,1-*f*][1,2,4]triazine-6-carbothioate derivatives **(8a-j).**

Anti-inflammatory activity of O-methyl 4-((substituted)amino)-5-methylpyrrolo[2,1-*f*] [1,2,4] triazine-6-carbothioate derivatives **(8a-j)** derivatives initially at 0-time interval no change in paw

edema was observed. Subsequently at 30 min interval, no significant variation in paw edema observed for test samples. At 60-minute time break, all the compounds showed good action of suppression of the paw edema. At 120-min time break **8a, 8b, 8e, 8f, 8g** and **8j** showed good action when compared to remaining test samples. Finally, results observed at 180 minutes' time break **8a, 8b, 8e, 8f, 8g** and **8j** samples showed comparable to the standard as shown in figure 7.

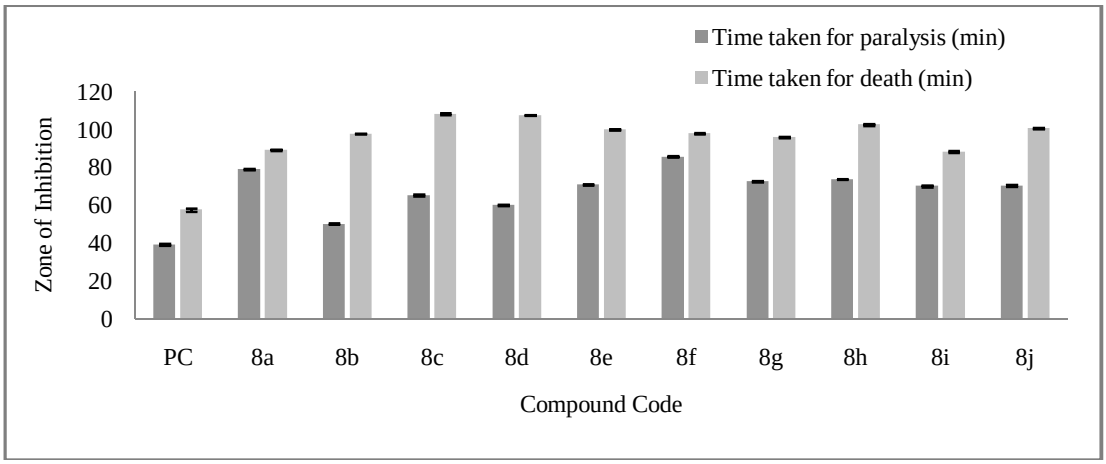


Figure 6. Anthelmintic activity values of O-methyl 4-((substituted)amino)-5-methylpyrrolo [2,1-*f*][1,2,4]triazine-6-carbothioate derivatives **(8a-j).**

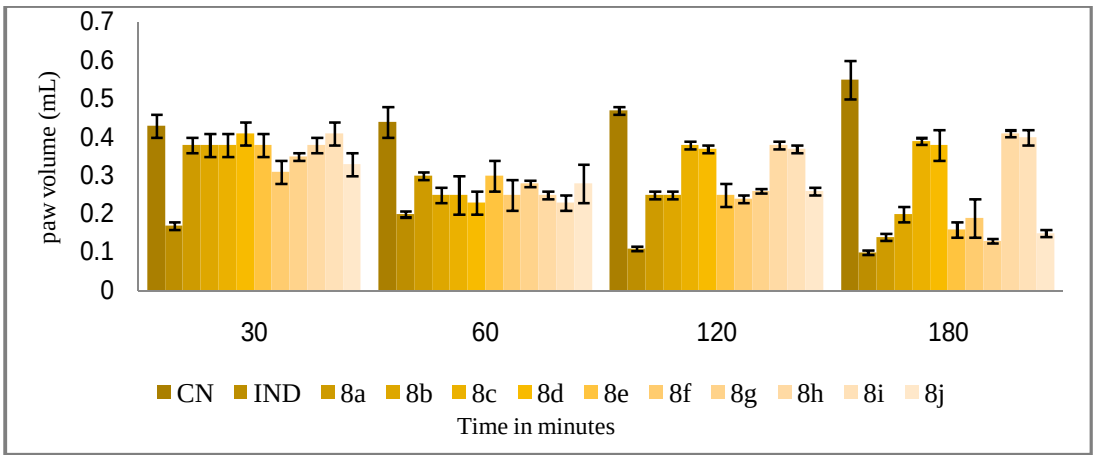


Figure 7. Anti-inflammatory activity values of O-methyl 4-((substituted)amino)-5-methylpyrrolo [2,1-f][1,2,4]triazine-6-carbothioate derivatives **(8a-j)**.

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APPLICATION

This study presents a very good platform for future researchers in synthesis of potent pharmacophores with specific potency.

CONCLUSION

Spectral evidences corroborate the assigned structural formula of the synthesized title compounds without any indistinctness. Further, based on the observed results of antibacterial and anti-inflammatory potency evaluation it is clear that synthesized molecules **8a, 8b, 8e, 8f, 8g** and **8j** are potent antibacterial and antifungal agents.

REFERENCES

[1]. Onofrio Migliara, Salvatore Petruso, Vincenzo Sprio, Synthesis of a new bridgehead nitrogen heterocyclic system. Pyrrolo [2,1-*f*]-1,2,4-triazine derivatives. *J. Heterocyclic. Chem.*, **1979**, 16(5), 833-834.

[2]. T. John Hunt, Toomas Mitt, Robert Borzilleri, Johnni Gullo-Brown, Joseph Fargnoli, Brian Fink, Wen-Ching Han, Steven Mortillo, Gregory Vite, Barri Wautlet, Tai Wong, Chiang Yu, Xiaoping Zheng, Rajeev Bhide, Discovery of the Pyrrolo[2,1-*f*][1,2,4]triazine Nucleus as a New Kinase Inhibitor Template, *J. Med. Chem.*, **2004**, 47(16), 4054-4059.

[3]. R. Gregory Ott, J. Gregory Wells, V. Tho Thieu, R. Matthew Quail, G. Joseph Lisko, F. Eugen Mesaros, E. Diane Gingrich, K. Arup Ghose, Weihua Wan, Lihui Lu, Mangeng Cheng, S. Mark Albom, S. Thelma Angeles, Zeqi Huang, D. Lisa Aimone, A. Mark Ator, A. Bruce Ruggeri, D. Bruce Dorsey, 2,7-Disubstituted-pyrrolo[2,1-*f*][1,2,4]triazines: New Variant of an Old Template and Application to the Discovery of Anaplastic Lymphoma Kinase (ALK) Inhibitors with in Vivo Antitumor Activity, *J. Med. Chem.*, **2011**, 54(18), 6328-6341.

[4]. Tho Thieu, A. Joseph Sclafani, V. Daniel Levy, Andrew McLean, J. Henry Breslin, R. Gregory Ott, P. Roger Bakale, D. Bruce Dorsey, Discovery and Process Synthesis of Novel 2,7-Pyrrolo[2,1-*f*][1,2,4]triazines, *Org. Lett.*, **2011**, 13(16), 4204-4207.

[5]. Z. El-Gendy, J. M. Morsy, H. A. Allimony, W. R. Ali, R. M. Abdel-Rahman. Synthesis of heterobicyclic nitrogen systems bearing the 1,2,4-triazine moiety as anti-HIV and anticancer drugs, part III. *Pharmazie*, **2001**, 56(5), 376-383.

[6]. W. D Schmitz, A.B Brenner, J. J Bronson, J. L Ditta, C. R Griffin, Y. W Li, N. J Lodge, T. F Molski, R. E Olson, X. Zhuo, J. E Macor. 5-Arylamino-1,2,4-triazin-6(1*H*)-one CRF_1 receptor antagonists, *Bioorg. Med. Chem. Lett,*. **2010**, 20, 3579-3583.

[7]. J. Hynes, A. J. Dyckman, S. Lin, S.T. Wrobleski, H. Wu, K.M. Gillooly, S. B. Kanner, H. Lonial, D. Loo, K.W. McIntyre, S. Pitt, D. R. Shen, D. J. Shuster, X. Yang, R. Zhang, K. Behnia, H. Zhang, P. H. Marathe, A. M. Doweyko, J. S. Tokarski, J. S. Sack, M. Pokross, S. E. Kiefer, J. A. Newitt, J. C. Barrish, J. Dodd, G. L. Schieven, K. Leftheris, Design, Synthesis, and Anti-inflammatory Properties of Orally Active 4-(Phenylamino)-pyrrolo[2,1-*f*][1,2,4]triazine p38α Mitogen-Activated Protein Kinase Inhibitors, *J. Med. Chem.*, **2008**, 51, 4-16.

[8]. K. Sztanke, J. Rzymowska, M. Niemczyk, I. Dybala, A. E. Koziol, Novel derivatives of methyl and ethyl 2-(4-oxo-8-aryl-2*H*-3,4,6,7-tetrahydroimidazo[2,1-*c*][1,2,4]triazin-3-yl)acetates from biologically active 1-aryl-2-hydrazinoimidazolines: Synthesis, crystal structure and antiproliferative activity, *Eur. J. Med. Chem.*, **2006**, 41, 1373-1384.

[9]. T. Kato, M. Nakata, M. Ide, K. Saito, T. Yoshida, T. Awaya, T. Heike, Efficacy and tolerability of topiramate, lamotrigine, and levetiracetam in children with refractory epilepsy, *No Hattatsu Brain Dev.*, **2015**, 47(5), 354-359.

[10]. S. A Patil, B. A Otter, R. S Klein, 4-Aza-7,9-dideazaadenosine, a New Cytotoxic Synthesis *C*-Nucleoside Analogue of Adenosine, *Tetrahedron Lett.* **1994**, 35, 5339-5342.

[11]. N. Subhakara Reddy, A. Srinivas Raoa, M. Adharvana Chari, V. Ravi Kumara, V. Jyothyc, V. Himabindu, Synthesis and antibacterial activity of sulfonamide derivatives at C-8 alkyl chain of anacardic acid mixture isolated from a natural product cashew nut shell liquid (CNSL), *J. Chem. Sci.*, **2012**, 124(3), 723-726.

[12]. M. Chester Himel, G. Wissam Aboul-Saad, U. K. Solang, Fluorescent analogs of insecticides and synergists. Synthesis and reactions of active-site-directed fluorescent probes, *J. Agr. Food Chem.*, **1971**, *19*(6), 1175-1180.

[13]. Ahmed Hanafy, Jun Uno, Hiroki Mitani, Yingqian Kang, Yuzuru Mikami. *In Vitro* Antifungal Activities of Sulfa Drugs against Clinical Isolates of *Aspergillus* and *Cryptococcus* Species, *Jpn. J. Med. Mycol.*, **2007**, 48(1), 47-50.

[14]. Zhao Yan-fang, Feng Run-liang, Liu Ya-jing, Zhang Yi-kun, Gong Ping. Synthesis and *in vitro* Anti-hepatitis B Virus Activity of Some Ethyl 5-Hydroxy-4-substituted Aminomethyl-2-sulfinylmethyl-1*H*-indole-3-carboxylates, *Chem. Res. Chinese Universities*, **2010**, 26(2), 272-277.

[15]. S. Guniz Kucukguzel, Inci Coskun, Sevil Aydin, Goknur Aktay, Sule Gursoy, Ozge Cevik, Amartya Basu, T. Tanaji Talele, Synthesis and Characterization of Celecoxib Derivatives as Possible Anti-Inflammatory, Analgesic, Antioxidant, Anticancer and Anti-HCV Agents, *Molecules*, **2013**, 18(3), 3595-3641.
[16]. M. Mostafa Ghorab, A. Fatma Ragab, I. Helmy Heiba, M. Hebaallah Agha, Synthesis of Some Novel Sulfonamides Containing Biologically Active Alkanoic Acid, Acetamide, Thiazole, and Pyrrole Moieties of Expected Antitumor and Radiosensitizing Activities, *J. Basic. Appl. Chem.*, **2011**, 1(2), 8-14.
[17]. S. Mansour Al-Said, M. Mostafa Ghorab, S. Mohammed Al-Dosari, M. Mostafa Hamed. Synthesis and *in vitro* anticancer evaluation of some novel hexahydroquinoline derivatives having a benzenesulfonamide moiety, *Eur. J. Med. Chem.*, **2011**, 46, 201-207.
[18]. Kamlesh Kumar Sahu, Veerasamy Ravichandran, Vishnu Kant Mourya, Ram Kishore Agrawal. QSAR analysis of caffeoyl naphthalene sulfonamide derivatives as HIV-1 integrase inhibitors, *Med. Chem. Res.*, **2007**, 15(7), 418-430.
[19]. P. J. Vora, A. G. Mehta, Russ. Synthesis, Characterization and Antimicrobial Efficacy of Quinoline Based Compound,. *IOSR. J. Appl. Chem.*, **2012**, 1(4), 34-39.
[20]. Gretchen M. Schroeder, Xiao-Tao Chen, David K. Williams, David S. Nirschl, Zhen-Wei Cai, Donna Wei, John S. Tokarski, Yongmi An, John Sack, Zhong Chen, Tram Huynh, Wayne Vaccaro, Michael Poss, Barri Wautlet, Johnni Gullo-Brown, Kristen Kellar, Veeraswamy Manne, John T. Hunt, Tai W. Wong, Louis J. Lombardo, Joseph Fargnoli, Robert M. Borzilleri, Identification of pyrrolo[2,1-*f*][1,2,4]triazine-based inhibitors of Met kinase, *Bioorganic. Med. Chem. Lett.*, **2008**, 18, 1945-1951.
[21]. Jonas Verhoeven, B. Narendraprasad Reddy, Lieven Meerpoel, Jan Willem Thuring, Guido Verniest, Synthesis and transformations of pyrrolo[1,2-*a*][1,3,5]-triazines, *Tetrahedron Lett*. **2018**, 59(52), 4537-4539.
[22]. Sunny Abraham, Michael J. Hadd, Lan Tran, Troy Vickers, Janice Sindac, Zdravko V. Milanov, Mark W. Holladay, Shripad S. Bhagwat, Helen Hua, Julia M. Ford Pulido, Merryl D. Cramer, Dana Gitnick, Joyce James, Alan Dao, Barbara Belli, Robert C. Armstrong, Daniel K. Treiber, Gang Liu. Novel series of pyrrolotriazine analogs as highly potent pan-Aurora kinase inhibitors, *Bioorganic. Med. Chem. Lett.*, **2011**, 21, 5296-5300.
[23]. Robert M. Borzilleri, Tracy Gerhardt, John Tokarski, Zhen-wei Cai, Bindu Goyal, Viral Vyas, Christopher Ellis, John T. Hunt, Barri Wautlet, Joseph Fargnoli, Steven Mortillo, Xioping Zheng, Aberra Fura, Ligang Qian, Rajeev S. Bhide. Synthesis and SAR of 4-(3-hydroxyphenylamino)pyrrolo[2,1-*f*][1,2,4]triazine based VEGFR-2 kinase inhibitors, *Bioorganic. Med. Chem. Lett.*, **2005**, 15, 1429-1433.
[24]. Zhen-wei Cai, Donna Wei, Robert M. Borzilleri, Ligang Qian, Amrita Kamath, Steven Mortillo, Barri Wautlet, Benjamin J. Henley, Robert Jeyaseelan, Sr, John Tokarski, John T. Hunt, Rajeev S. Bhide, Joseph Fargnoli and Louis J. Lombardo. Synthesis, SAR, and Evaluation of 4-[2,4-Difluoro-5-(cyclopropylcarbamoyl)phenylamino]pyrrolo[2,1-*f*] [1,2,4] triazine-based VEGFR-2 kinase inhibitors, *Bioorganic.Med. Chem. Lett.*, **2008**, 18, 1354-1358.
[25]. N. R Mohan, S. Sreenivasa, K. E. Manojkumar, T. Madhu Chakrapani Rao. Synthesis and in vitro antibacterial activity of some novel sulfonamide derivatives bearing 1,4-disbstituted1,2,4-oxadiazole Moiety, *J. Applicable Chem,*. **2013**, 2(4), 722-729.
[26]. K. E Manojkumar, S Sreenivasa, N. R Mohan, T. Madhuchakrapani Rao, T. Harikrishna, Synthesis, Characterization and Biological evaluation of novel substituted acid amides containing piperazine 1,2,4-Oxadiazole nucleus, *J. Applicable Chem*, **2013**, 2(4), 730-737.
[27]. K. E. Manojkumar, S. Sreenivasa, N. R. Mohan, P. A. Suchetan, C. G. Darshan Raj, H. Raja Naika, 2-[5-(2-fluorophenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzoic acid: Synthesis, Characterization and Pharmacological Evaluation, *J. Applicable Chem.*. **2014**, 3(1), 64-73.
[28]. N. R Mohan, S. Sreenivasa, K. E Manoj Kumar, H. C Anitha, T. Madhu Chakrapani Rao, B. S. Thippeswamy, K. Vijay. Synthesis, Characterization and In-Vitro Antimicrobial Study of Series of 1-((Substituted Aryl/Alkyl)Sulfonyl)-4Tosylpiperazines, *Indo American Journal of Pharm Research*, **2013**, 3(12), 1513-1520.

[29]. Karikere Ekanna Manojkumar, Swamy Sreenivasa, Nadigar Revanasiddappa Mohan, P. A. Suchetan, Chenna Govindaraju Darshan Raj, Hanuma Naika Raja Naika, 2-[5-(2-fluorophenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl] benzoic acid: Synthesis, Characterization and Pharmacological Evaluation, *J. Applicable Chem.*, **2014**, 3(1), 64-73.
[30]. B. J. Shankar, S. Sreenivasa, N. R. Mohan, Synthesis of Active Pharmaceutical Ingredient Derivatives and Their Anti-Inflammatory and Analgesic Activity Studies, *J. Applicable Chem.*, **2015**, 4(6): 1743-1750.
[31]. S. Cuzzocrea, B. Zingarelli, G. Costantino, A. Szabo, A. L. Salzman, A. P. Caputi, C. Szabo, Beneficial effects of 3-aminobenzamide, an inhibitor of poly (ADP-ribose) synthetase in a rat model of splanchnic artery occlusion and reperfusion, *Br J Pharmacol*, **1997**, 121, 1065-1074.
[32]. G. K. Dash, P. Suresh, D. M. Kar, S. Ganpatry, S. B. Panda. Evaluation of Evolvulus alsinoides Linn. For anthelmintic and antimicrobial activities, *J. Nat. Rem.*, **2002**, 2, 182-185.
[33]. Nadigar R. Mohan, Swamy Sreenivasa, Karikere E. Manojkumar, Tadimety M. C. Rao, Boreddy S. Thippeswamy, Parameshwar A. Suchetan, Synthesis, Antibacterial, Anthelmintic and Anti-Inflammatory Studies of Novel Methylpyrimidine Sulfonyl Piperazine Derivatives, *J. Braz. Chem. Soc.*, **2014**, 25(6), 1012-1020.