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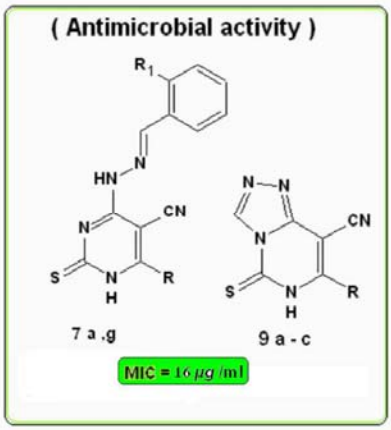

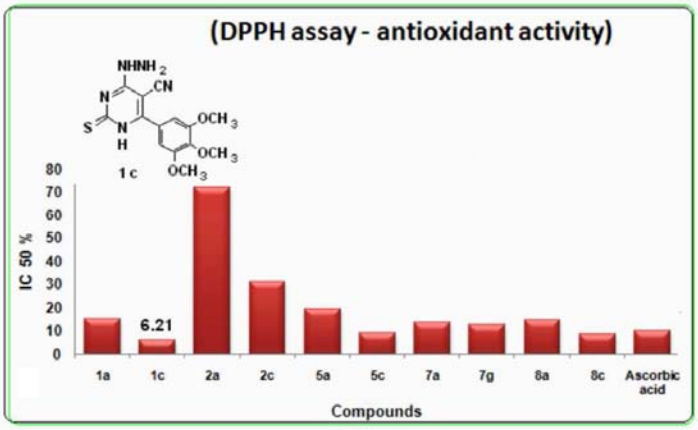

►A series of novel 6-aryl-5-cyano thiouracils (**2a-c** to**11a-c**) was synthesized from 6-aryl-4-hydrazino-2-thioxo-1, 2-dihydropyrimidine-5-carbonitriles (**1a-c**).

►Antimicrobial and antioxidant studies were performed. ► Potent activities were observed.

Synthesis, antimicrobial, antioxidant activities of novel 6-aryl- 5-cyano thiouracil derivatives

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ABSTRACT

A series of 6-aryl-5-cyano thiouracil derivatives **(2a-c** to**11a-c)** was synthesized from 6-aryl-4-hydrazino-2-thioxo-1,2-dihydropyrimidine-5-carbonitriles **(1a-c)** .The products were characterized by analytical and spectral data (IR,^{1}HNMR,^{13}CNMR and mass spectra).All compounds were screened for their *in-vitro* antibacterial and antifungal activities. Compounds **7a**,**7g** and **9a-c** showed pronounced antimicrobial activity than standards. Some of the newly synthesized compounds were evaluated for antioxidant activity. Compounds **1c**,**5c** and **8c** displayed promising free radical scavenging activity and found to be more potent than standard, ascorbic acid (vitamin C).

Keywords: Synthesis, 6-aryl -5-cyano thiouracils, antibacterial, antifungal, antioxidant activity, radical scavenging.

Graphical abstract

1. Introduction

Pyrimidine constitutes an important component of nucleic acid and it is used as building blocks in pharmaceutics for the synthesis of antiviral, anticancer,antioxidant,antiinflammatory, antibacterial and antifungal agents[1-7].Similarly, the related thiouracil derivatives are potential therapeutics as antiviral, antioxidant, anticancer and antimicrobial agents [8-11]. Moreover, a literature survey revealed that the thiouracil carbonitrile ring system has occupied a marked position in the design and synthesis of novel chemotherapeutic agents with remarkable antitumor **I**, HCV inhibitors**II** and antimicrobial activities **III**(Fig.1)[12-14].Reports from our laboratory[15] and from others [16] revealed the synthesis of 4-hydrazinothiopyrimidine-5-carbonitriles from 4-chloro derivatives. These hydrazino derivatives exerted promising antibacterial, antifungal and anticancer activities [17-19]. In addition, the reactions of hydrazino pyrimidines with formic acid, trietylorthoformate(TEOF)and CS_2(one carbon donor moieties) afforded the corresponding triazolopyrimidines[20-25],which are known to exhibit interesting Pharmaceutical activities [26, 27]. In the light of the aforementioned facts, and in continuation for our interest in the synthesis of biologically active heterocyclic compounds, we report herein the synthesis and biological evaluation of novel 6-aryl-5-cyano thiouracil derivatives as antimicrobial and antioxidant agents.

Fig.1. Structures of some potent 5-cyano -2-thiouracils.

2. Results and discussion

Chemistry

The synthetic pathways adopted for the preparation of the desired new compounds are illustrated in charts 1, 2.

Synthesis of hydrazino pyrimidines **1a-c** was achieved through two synthetic pathways as previously reported from our laboratory [15], the first of which was the reaction of 6-aryl-5-cyano-2-thiouracils with $PoCl_3/PCl_5$ to obtain the chloro derivatives. Reaction of the latter compounds with hydrazine hydrate (99%) in methanol afforded **1a-c**. Hydrazino derivatives**1a-c** were used as key compounds for this study and for synthesis of other fused heterocycles. In this investigation ,and in continuation for our previous work [28-31] in the synthesis of different fused triazolo pyrimidines, refluxing of compounds **1a-c** with acetic anhydride ,formic or acetic acid ,ethyl chloroformate (ECF) and carbon disulfide ; gave 7-aryl-3-methyl-5-thioxo-5,6-dihydro-[1,2,4]triazolo [4,3-c] pyrimidine-8-carbonitriles **2a-c** ,7-aryl-5-thioxo-5,6-dihydro[1,2,4]triazolo[1,5-c]-pyrimidine-8-carbonitriles **3a-c**,7-aryl-2-methyl-5-thioxo-5,6-dihydro [1,2,4]triazolo [1,5-c]-pyrimidine-8-carbonitriles **4a-c**,7-aryl-3-oxo-5-thioxo-2,3,5,6-tetrahydro[1,2,4]-triazolo[4,3-c]pyrimidine-8-carbonitriles **5a-c** and 7-aryl-3,5-dithioxo-2,3,5,6-tetrahydro[1,2,4]triazolo [4, 3-c]pyrimidine-8-carbonitriles**6a-c**,respectively[16,25,32,33]as revealed in scheme1. Furthermore, reaction of 4-hydrazinopyrimidines with different aromatic aldehydes in ethanol afforded the corresponding 4-[(2E)-2-substituted (benzylidene) hydrazino]-6-(aryl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitriles**7a-i**[34,35],while cyclocondensation of compounds**1a-c** with diethyloxalate,triethylorthoformate and acetyl acetone afforded carbonitriles**(8-10)a-c**,respectively[12,25,19].Finally,the reaction of compounds **1a-c** with aqueous solution of sodium nitrite in HCl at 0-5˚C afforded triazolo[4,3-c]pyrimidine-8-carbonitriles**11a-c**[36].The structures of new compounds were confirmed by MS,IR,^{1}H NMR,^{13}CNMR as well as elemental analysis. IR spectra of compounds **1a-c** showed CN bands at 2200-2230 $cm^{-1}$and two absorbance bands at 3490 -3200 cm^{-1}

corresponding $NHNH_2$ as reported [16] .^{1}HNMR spectra showed three singlet of $NHNH_2$ around 5.1 and 5.7 ppm. The ^{1}HNMR spectra of compounds **2a-c** showed a singlet signal at 2.3-2.5 ppm corresponding to CH_3 of triazole ring. The ^{13}C NMR spectra displayed signals for CH_3 aliphatic carbon and for C=N carbon of triazole ring. The^{1}HNMR spectra for **3a-c** revealed singlet signals for CH=N and one NH instead of three signals like in **1a-c** ,for **4a-c** signals for CH_3 and one NH. The ^{13}CNMR spectra of products were compatible with the proposed structure. Also,the structures of **5a-c** were established on the basis of IR, which showed the presence of C=O while ^{1}H NMR spectra revealed two signal for NH proton. The ^{13}CNMR spectra displayed signals for C=O carbon. The ^{1}HNMR spectra of **6a-c** showed two singlet signals for NH proton. Moreover, the^{13}CNMR spectra showed the presence of two C=S signals corresponding to C=S of thiouracil and triazole ring. Compounds**7a-i** and **8a-c** were confirmed by spectral data and the mass spectra studies of these compounds gave additional evidence for the proposed structures.^{1}HNMR for **9a-c** showed singlet signals for CH=N and one NH .The^{13}CNMR spectra displayed signals for C=N carbon. Also, MS spectra gave their molecular ion peaks.The ^{1}HNMR of **10a-c** showed two singlet signals at 2-2.53 ppm corresponding to two CH_3 group and only one singlet corresponding to NH. The IR and ^{13}CNMR spectra of products **10a-c** were compatible with the proposed structure. The IR spectra of **11a-c** showed the absence of $NHNH_2$ as well as the presence of C=N and ^{1}H NMR showed a singlet signal for NH proton.

Scheme 1

Scheme 1. Synthetic Pathway for the preparation of compounds **1 ~ 6 (a - c)**.

Scheme 2

Scheme 2. Synthetic Pathway for the preparation of compounds **7 (a-i) ~11 (a - c)**.

Biological results and discussion

Antimicrobial studies

Antimicrobial studies (Table I) showed that compounds **7a-i** and **9a-c** possess a pronounced antimicrobial activity against *Staphylococcus aureus ,Bacillus cereus, Escherichia coli,Candida albicans* and *Aspergillus flavus* compared to the reference drugs, while compounds **1a-c**,**10a-c** and **11a-c** showed marked antibacterial activity against SA,BC and EC but didn't show any antifungal activity except **1a** showed moderate activity against *Aspergillus flavus*. Compounds **6a-c** exhibited high activities also towards BC ,EC and CA,while inactive against SA and AF, compounds **8a-c** towards *Staphylococcus aureus* and *Aspergillus flavus* only. Compounds **5a-c** possess high activities towards *Staphylococcus aureus* only.Compounds**2a-c**,**3a-c** and **4a-c** were either inactive or weakly active against the tested microorganisms.

The present study revealed that conversion of 4-hydrazino pyrimidines to hydrazones **(7a-i)** or triazolo[4,3-c] pyrimidines **(9a-c)** caused a pronounced inhibition effect against Gram-positive (*Staphylococcus aureus, Bacillus cereus*), Gram-negative (*Escherichia coli*) bacteria and fungi (*Candida albicans* and *Aspergillus flavus)*.On the other hand, increased antibacterial activity was achieved by cyclization to triazolo[4,3-c]pyrimidines , pyazolopyrimidines or tetrazolo pyrimidines as in **6**,**10** and **11a-c** ,while cyclization to triazolo[4,3-c] pyrimidines or pyrimidotriazines as in **5**, **8a-c** increased antibacterial activity only against *Staphylococcus aureus* or *S.aureus* and *Asp.flavus*, respectively.

However, cyclization to triazolo [1,5-c]pyrimidines or triazolo[4,3-c] pyrimidines as in **3**,**4a-c** or **2a-c** either decreased or diminished antimicrobial activity , respectively (see Inline Supplementary,Figs.S1-S10).

The structure activity relationship suggested that conversion of thiouracils to hydrazones or triazolo [4, 3-c] pyrimidines showed higher antibacterial and antifungal activities than other derivatives.

Table 1 and Figure 2,3.

Table I. Antimicrobial activity results of newly synthesized compounds, expressed as inhibition zone diameter (mm) and MIC (μg/ml) with the standard drugs.

Fig.2: Antibacterial activity (Gram +ve,-ve) of synthesized compounds.

Fig.3: Antifungal activity of synthesized compounds.

Antioxidant evaluation

The DPPH radical scavenging ability of some synthesized compounds was evaluated and compared to those of the well-known antioxidant ascorbic acid (table II).

Our compounds were able to reduce DPPH in a concentration-dependent manner. The tested samples were statistically different ($P < 0.05$, Kruskal-Wallis test) over the dose range used. The maximum scavenging activity of DPPH was produced with compounds **1c**,**5c** and **8c** with IC_{50} of 6.21,9.34,8.55 µg/mL, respectively **(Fig.4,5)**.On critical overview of synthesized compounds, it has been found that compounds with electron donating group ($-OCH_3$) on phenyl ring exhibited potent antioxidant activity.

Table II and Figure 4, 5.

Table II. IC_{50} values (in **µg/mL**) for DPPH scavenging ability of the compounds

Fig.4. Screening of antioxidant activity by the DPPH assay shows that **1c**, **5c** and **8c** have highest activity (more potent than the reference drug (RF). Each value represents a mean ± SEM (n= 3).

Fig.5. DPPH radical scavenging activity of compound 1c. Each value represents a mean ± SEM (n = 3).

Conclusions

Evaluation of the new compounds established that the most promising antimicrobial compounds are 4- [(2E)-2-benzylidene- hydrazino]-6-(4-fluoro-phenyl)-2-thioxo-1, 2, 3, 4-tetrahydropyrimidine-5-carbonitrile **(7a)**, [(2E)-2-(4-methoxybenzylidene)-hydrazino] -6-(4-fluoro-phenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile**(7g)**,7-(4-fluo-oro-phenyl)-5-thioxo-5,6-dihydro[1,2,4] triazolo[4,3-c]- pyrimidine-8-carbonitrile(**9a**), 7-(4-Bromo-phenyl)-5-thioxo-5,6-dihydro[1,2,4]triazolo[4,3-c]-pyrimidine-8-carbonitr-ile **(9b)** and 5-thioxo-7-(3,4,5-trimethoxy-phenyl)-5,6-dihydro- [1,2,4] triazolo[4,3-c]-pyrimidine-8-carbonitrile(**9c**).Compounds **1c**,**5c** and **8c** were found to possess promising antioxidant activity when compared with standard ascorbic acid (vitamin C).

3. Experimental

3.1. Chemistry

All melting points are uncorrected and measured using Electro- thermal IA 9100 apparatus (Shimadzu, Japan). IR spectra were recorded as potassium bromide pellets on a Perkin-Elmer 1650 spectrophotometer (USA), Faculty of Science, Cairo University, Cairo, Egypt.^{1}HNMR and ^{13}C-NMR spectra (see Supplementary data) were determined on a Varian Mercury (300 MHz) spectrometer (Varian UK) and chemical shifts were

expressed as ppm against TMS as internal reference (Faculty of Science, Cairo University, Cairo, Egypt). Mass spectra were recorded on 70 eV EI Ms-QP 1000 EX (Shimadzu, Japan), Faculty of Science, Cairo University, and Cairo, Egypt. Microanalyses were operated using Vario, Elementar apparatus (Shimadzu, Japan), Organic Microanalysis Unit, Faculty of Science, Cairo University, Cairo, Egypt and the results were within the accepted range (0.40) of the calculated values. Column Chromatography was performed on (Merck) Silica gel 60 (particle size 0.06-0.20 mm).

3.2.General method for preparation of I a - c

Synthesis of compounds was performed according to the literature [16].A mixture of the appropriate thiouracil **a** (2.65 g,0.01 mol), **b** (2.90 g,0.01 mol) , **c** (3.26 g,0.01 mol) and hydrazine hydrate (20 mL, 99%) was refluxed in methanol (30 mL) for 20m,cooled , stirred for 24 h and poured onto ice - water. The solid obtained was filtered, dried and recrystallized from DMF/ water to yield compounds **1a- c**, respectively.

3.2.1.6-(4-Fluoro-phenyl)-4-hydrazino-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile

1a.Yield 65%,m.p.200-202˚C.IR (max /cm^{-1}): 3460-3200(NH_2,2NH),2200 (CN) ,1270 (C=S).^{1}HNMR(300MHz,DMSO-d_6):δ5.1-5.7(s,3H,NH,NH_2,D_2Oexchangeable),7.3-7.8 (m, 4H,Ar-H),10.3(s,1H,NH,D_2O exchangeable).^{13}CNMR(300MHz,DMSO-d_6):δ90.42, 114.17,115.38,125.27,131.26,157.96,159.57,161.82,175.71.MS (EI): m/z:261[M+](15.4 %).Anal. Calcd for $C_{11}H_8FN_5S$(261.27):C,50.57;H,3.09; N,26.80;S,12.27; Found: C,50. 42; H,2.90; N,26.93;S,12.43.

3.2.2.6-(4-Bromo-phenyl)-4-hydrazino-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile

1b. Yield 66%, m.p.190-192˚C.IR (max/cm^{-1}):3490-3264 (NH_2, 2NH), 2225(CN),1275 (C=S).^{1}H NMR (300MHz,DMSO-d_6):δ 5.2-5.7(s,3H,NH,NH_2, D_2O exchangeable), 7.3-7.8(m,4H,Ar-H),10.5(s,1H,NH,D_2O exchangeable).^{13}CNMR(300MHz,DMSO-d_6):δ87.9 ,117,122.3,128.4,131.7,133.9,164,169,182.MS(EI):m/z:321[M+](24.8%),323[M+2] (24.7%).Anal.Calcd for $C_{11}H_8BrN_5S$(320.97): C,41.01; H, 2.50; N,21.74; S,9.95; Found: C,41.18;H,2.64;N,21.62;S,10.02.

3.2.3.4-hydrazino-2-thioxo-6-(3,4,5-trimethoxy-phenyl)-1,2-dihydropyrimidine-5-carbo-nitrile **1c**.Yield 68%,m.p.215-217˚C.IR (max/cm^{-1}):3490-3300 (NH_2, 2NH),2230 (CN), 1268(C=S).^{1}HNMR (300 MHz, DMSO-d_6):δ 3.1- 3.7(s,9H,3OCH_3),5.1-5.7 (s, 3H,NH, NH_2,D_2Oexchangeable),6.6,6.8(s,2H,Ar-H),10.2(s,1H,NH,D_2O exchangeable).^{13}CNMR (300MHz,DMSO-d_6):δ55.4,55.7,85.8,115,117,129.2,132.4,160,163,167.5,183.MS (EI): m/z:333[M+](15.3%).Anal.Calcd for $C_{14}H_{15}N_5O_3S$ (333.36):C,50.44; H, 4.54;N,21.01; S,9.62;Found:C,50.58;H,4.42;N,21.10;S,9.77.

3.3.General method for preparation of 2 a - c

A mixture of the appropriate thiouracil derivative **1a - c** (0.01 mol) and acetic anhydride (30 mL) was heated under reflux for 2h.The solid obtained was filtered, dried and recrystallized from acetic acid to yield compounds **2a- c**, respectively.

3.3.1.7-(4-Fluoro-phenyl)-Aryl-3-methyl-5-thioxo-5, 6-dihydro-[1, 2, 4] triazolo [4, 3-c] pyrimidine-8-carbonitrile **2a**.Yield55%, m.p.170-172˚C.IR (max/cm^{-1}):3262(NH), 2216 (CN),1270(C=S),1598(C=N).^{1}H NMR (300 MHz,DMSO-d_6):δ2.3(s,3H,CH_3),6.9-7.9 (m,4H,Ar-H),9.1(s,1H,NH,D_2Oexchangeable).^{13}CNMR(300MHz,DMSO-d_6):δ20.5, 86.5,113.3,115.2,127.2,130.4,150.3,152.4,159.5,163.2,177.2.MS (EI):m/z:285[M+]

(40 %). Anal. Calcd for $C_{13}H_8FN_5S$ (285.29):C,54.73;H,2.83;N,24.55;S,11.24; Found:C,54.80; H,2.90; N,24.70;S,11.34.

3.3.2.7-(4-Bromo-phenyl)-3-methyl-5-thioxo-5,6-dihydro-[1,2,4]triazolo[4,3-c]pyrimidine-8-carbonitrile **2b**.Yield 53%,m.p.208-210˚C.IR (max/cm^{-1}):3250(NH),2220(CN), 1275(C=S),1595(C=N).^{1}HNMR(300MHz,DMSO-d_6):δ2.5(s,3H,CH_3),7-7.9(m,4H,Ar-H),9.4(s,1H,NH,D_2Oexchangeable).^{13}CNMR(300MHz,DMSO-d_6):δ20.3,92.6,114.05, 115.4,128.8,133.4,150.3,151.4,160.5,162,175.2.MS (EI): m/z:345[M +] (55.1%),347 [M+2](55.3%).Anal. Calcd for $C_{13}H_8BrN_5S$ (344.97):C,45.10;H,2.33;N,20.23;S,9.26 ;Found:C,45.30;H,2.50;N,20.40;S,9.34.

*3.3.3.3-Methyl-5-thioxo-7-(3,4,5-trimethoxy-phenyl)-5, 6-dihydro-[1,2,4] triazolo [4, 3-c]pyrimidine-8-carbonitrile***2c**.Yield 50%,m.p.223-225˚C.IR(max/cm^{-1}):3277(NH),2222 (CN),1277(C=S),1590(C=N).^{1}HNMR(300 MHz,DMSO-d_6):δ2.52(s,3H,CH_3),3.4-3.86 (s,9H,3OCH_3),6.82,7(s,2H,Ar-H),10.14(s,1H,NH,D_2O exchangeable.^{13}CNMR(300MHz ,DMSO-d_6):δ20.4,55.4,55.7,88.7,114,115.3,130.4,151.7,152,153.9, 159.3,168,178.MS (EI):m/z:357[M+](60%).Anal. Calcd for $C_{16}H_{15}N_5O_3S$(357.38):C,53.77;H,4.23;N,19.60 ;S,8.97;Found:C,53.80; H,4.30;N,19.77;S,9.04.

3.4. General method for preparation of 3a-c and 4a-c

A mixture of appropriate thiouracil derivative **1a- c** (0.01 mol) and formic or acetic acid (30 mL) was heated under reflux for 8h ,then cooled and poured onto ice - water. The solid obtained was crystallized from acetic acid to yield compounds **3**,**4a-c**, respectively.

3.4.1.7-(4-Fluoro-phenyl)-5-thioxo-5,6-dihydro[1,2,4]triazolo[1,5-c]-pyrimidine-8-car-

bonitril **3a**.Yield 45%,m.p.228-230˚C.IR(max/cm^{-1}):3225(NH),2216(CN),1270 (C=S), 1613(C=N).^{1}HNMR(300MHz,DMSO-d_6):δ8.60(s,1H,C_3-H),7-7.7(m,4H,Ar-H),9.2(s, 1H,NH,D_2O exchangeable).^{13}CNMR(300MHz,DMSO-d_6):82.3,115,117.2,127.4,130.1 ,149.7,151.2,161.5,166,176.2.MS(EI):m/z:271[M+](56%).Anal.Calcd for $C_{12}H_6FN_5S$ (271.27):C,53.13;H,2.23;N,25.82;S,11.82;Found:C,53.33; H,2.41;N,25.95; S, 11.94.

3.4.2.7-(4-Bromo-phenyl)-5-thioxo-5,6-dihydro[1,2,4]triazolo[1,5-c]-pyrimidine-8-car-Bonitrile **3b**.Yield47%,m.p.218-220˚C.IR(max/cm^{-1}):3322(NH),2220(CN),1270(C=S), 1612(C=N).^{1}HNMR(300MHz,DMSO-d_6):δ8.5(s,1H,C_3-H),7.2-7.6(m,4H,Ar-H),9.5(s, 1H,NH,D_2Oexchangeable).^{13}CNMR(300MHz,DMSO-d_6):δ85.1,117.2,122.3,128.4,131. 7,148.5,153.1,163,165.1,175.7.MS(EI):m/z:331[M+](59.2%),333[M+2] (59.4%).Anal. Calcd for $C_{12}H_6BrN_5S$ (330.95):C,43.39;H,1.82;N,21.08;S,9.65;Found: C,43.51; H,1.94; N,21.26;S,9.78.

3.4.3.5-thioxo-7-(3,4,5-trimethoxy-phenyl)-5,6-dihydro[1,2,4]triazolo[1,5-c]-pyrimidine -8-carbonitrile **3c**.Yield 45%, m.p. 208-210˚C.IR(max/cm^{-1}):3230(NH),2222(CN),1270 (C=S),1609(C=N).^{1}HNMR(300MHz,DMSO-d_6):δ3-3.8(s,9H,3OCH_3),8.63(s,1H,C3-H), 7,7. 5(s,2H,Ar-H),9.3(s,1H,NH,D_2O exchangeable).^{13}CNMR(300 MHz,DMSO-d_6):δ 55.4,55.7, 80.9,115.1,117.2,129.2,132.4,148.5,152.2,161.8,165.3,176.1.MS(EI):m/z:343 [M+](42%).Anal.Calcd for $C_{15}H_{13}N_5O_3S$ (343.36):C,52.47;H,3.82;N,20.40; S,9.34; Found: C,52.58; H,3.97; N,20.61; S,9.44.

3.4.4.7-(4-Fluoro-phenyl-2-methyl-5-thioxo-5,6-dihydro[1,2,4]triazolo[1,5-c]-pyrimid-

ine-8-carbonitrile **4a**.Yield52%,m.p.233-235˚C. IR (max/cm^{-1}):3225(NH),2216(CN), 1270(C=S),1607(C=N).^{1}HNMR(300MHz,DMSO-d_6):δ2.2(s,3H,CH_3),7.1-7.8(m,4H, Ar-H),9.8(s,1H,NH,D_2Oexchangeable).^{13}CNMR(300MHz,DMSO-d_6):*δ20.2,* 84.3,113, 117.2,124.4,127.1,143.7,150.3,162.5,165,182.MS (EI): m/z: 285[M+](60%).Anal. Calcd for $C_{13}H_8FN_5S$ (285.29):C,54.73;H,2.83;N,24.55;S,11.24;Found:C,54. 88;H,2.94;N, 24. 68; S,11.35.

3.4.5.7-(4-Bromo-phenyl-2-methyl-5-thioxo-5,6-dihydro[1,2,4]triazolo[1,5-c]-pyrimid-ine-8-carbonitrile **4b**.Yield 54%, m.p.222-224˚C.IR (max/cm^{-1}):3333(NH),2216(CN), 1270(C=S),1602(C=N).^{1}H NMR(300MHz,DMSO-d_6):δ 2.3(s,3H,CH_3),7.2-7.7(m,4H, Ar-H),9.3(s,1H,NH,D_2O exchangeable).^{13}CNMR(300 MHz,DMSO-d_6):δ20.2,82.5, 117.2,122.3,128.4,131.7,140.2,151.2,160.3,166.9,182.MS EI): m/z:345[M+] (62.1%), 347[M+2](62.3%).Anal.Calcd for $C_{13}H_8BrN_5S$ (344.97): C,45.10;H, 2.33; N,20.23; S, 9.26; Found: C,45.22; H,2.55; N,20.36; S,9.39.

3.4.6.2-Methyl-5-thioxo-7-(3,4,5-trimethoxy-phenyl)-5,6-dihydro[1,2,4]triazolo[1,5-c]-Pyrimidine-8-carbonitrile **4c**.Yield 55%,m.p.228-230˚C.IR(max/cm^{-1}):3240(NH), 2216 (CN),1270(C=S),1605(C=N).^{1}HNMR(300MHz,DMSO-d_6):δ2.2(s,3H,CH_3),3-3.7(s,9H ,3OCH_3),7,7.4(s,2H,Ar-H),9.2 (s,1H,NH,D_2O exchangeable).^{13}CNMR(300 MHz,DMSO -d_6):δ20.3,55.4,55.6,81.1,115.1,117,129.2,132.4,148.5,151.9,162.3,166.8,182.MS(EI): m/z:357 [M+](71%).Anal.Calcd for $C_{16}H_{15}N_5O_3S$ (357.38):C,53.77; H,4.23; N,19.60; S, 8.97; Found: C,52.89;H,4.35;N,19.72;S,9.10.

3.5. General method for preparation of 5a - c

A mixture of appropriate thiouracil derivative **1a - c** (0.01 mol) and ethylchloroformate (0.02 mol) in pyridine (30 mL) was heated under reflux for 12h and then poured on HCl. The solid obtained was crystallized from dimethyl formamide to yield compounds **5a- c**, respectively.

3.5.1.7-(4-Fluoro-phenyl)-3-oxo-5-thioxo-2,3,5,6-tetrahydro[1,2,4]-triazolo [4,3-c]pyrimidine-8-carbonitrile **5a**.Yield 51%,m.p.178-180˚C. IR(max/cm^{-1}):3430-3100(NH), 2215(CN),1661(C=O),1625(C=N).^{1}HNMR(300MHz,DMSO-d_6):δ7.1-7.5(m,4H,Ar-H) ,8.1,9.2(s,2H,NH,D_2Oexchangeable).^{13}CNMR(300MHz,DMSO-d_6): δ77,113,117,124.4, 143.7,147.8,155,165,170,178.MS (EI):m/z:287[M+](35%).Anal.Calcd for $C_{12}H_6FN_5OS$ (287.27):C,50.17; H, 2.11;N,24.38;S,11.16; Found:C,50.29;H,2.25;N,24.49;S,11.27.

3.5.2.7-(4-Bromo-phenyl)-3-oxo-5-thioxo-2,3,5,6-tetrahydro[1,2,4]-triazolo[4,3-c]pyrimidine-8-carbonitrile **5b**.Yield 50%,m.p.173-175˚C.IR (max/cm^{-1}):3450-3190(NH), 2225(CN),1673(C=O),1635(C=N).^{1}HNMR(300MHz,DMSO-d_6):δ7.3-7.8(m,4H,Ar-H), 8.3,9(s,2H,NH,D_2Oexchangeable).^{13}CNMR(300MHz,DMSO-d_6):δ75, 117,122.1,128.4, 133.2,147.8,158,168,170,177.MS(EI):m/z:347[M+](43.3%),349 [M+2] (43.5%).Anal. Calcd for $C_{12}H_6BrN_5OS$ (346.95): C,41.40; H,1.74; N,20.11; S, 9.21; Found: C,41.55; H,1.83;N,20.23;S,9.35.

3.5.3.3-Oxo-5-thioxo-7-(3, 4, 5-trimethoxy-phenyl)-2, 3, 5, 6-tetrahydro [1, 2,4]-triazolo [4, 3-c] pyrimidine-8-carbonitrile **5c**.Yield 53%, m.p.185-187˚C.IR(max/cm^{-1}):3400-

3180(NH),2225(CN),1677(C=O),1635(C=N).^{1}HNMR(300MHz,DMSO-d_6):δ3-3.7(s,9H ,3OCH_3),7.1,7.6(s,2H,Ar-H),8.1,9.2(s,2H,NH,D_2Oexchangeable).^{13}CNMR(300MHz, DMSO-d_6):δ55.55,55.75,78.04,104.82,114.05,122,130.45,146.79,152,157.98,159.79, 178.04. MS (EI):m/z:359 [M+](40%).Anal.Calcd for $C_{15}H_{13}N_5O_4S$(359.35):C,50.13;H, 3.65; N,19.49; S,8.92; Found: C,50.26; H,3.77; N,19.55; S,9.10.

3.6. General method for preparation of 6a - c

To an ice cold solution of appropriate thiouracil derivative **1a-c** (0.01 mol) and KOH (0.01 mol) in ethanol (20 mL) was added dropwise with stirring CS_2 (10mL), then the reaction mixture was refluxed on a water-bath for 5h. The reaction mixture cooled, poured onto ice -water, neutralized with HCl, filtered, crystallized from ethanol to yield compounds **6 a- c**, respectively.

3.6.1.7-(4-Fluoro-phenyl)-3,5-dithioxo-2,3,5,6-tetrahydro[1,2,4]triazolo[4,3-c]pyrimid-ine-8-carbonitrile **6a**.Yield 54 %, m.p.240-242˚C.IR (max/cm^{-1}):3333-3160 (NH),2227 (CN),1650(C=S),1580(C=N).^{1}HNMR(300MHz,DMSO-d_6):δ6.6-7.6(m,4H,Ar-H),9.1,10 (s,2H,NH,D_2O exchangeable).^{13}CNMR(300MHz,DMSO-d_6):δ78.7,113.8,117.2,124.4, 127.1,143.7,166,173,178,185.MS (EI) :m/z: 303[M+](32%).Anal.Calcd For $C_{12}H_6FN_5S_2$ (303.33):C,47.51;H,1.99;N,23.09;S,21.14;Found:C,47.73;H,2.12; N, 23.28; S,21.27.

3.6.2.7-(4-Bromo-phenyl)-3,5-dithioxo-2,3,5,6-tetrahydro[1,2,4]triazolo[4,3-c]pyrimid-ine-8-carbonitrile **6b**.Yield50 %,m.p.230-232˚C. IR (max/cm^{-1}):3335-3180 (NH),2225 (CN),1649(C=S),1585(C=N).^{1}H NMR (300MHz, DMSO-d_6): δ6.8-7.9(m, 4H,Ar-H),

,9.3,10.1(s,2H,NH,D_2O exchangeable).^{13}CNMR(300MHz,DMSO-d_6): 86.78,110.27, 115.28,128.51,130.04,147.47,164.04,172.04,175.72,186.01. MS(EI): m/z:363[M+] (27.2%),365[M+2](27.3%).Anal.Calcd for $C_{12}H_6BrN_5S_2$(362.92):C,39.57;H,1.66;N, 19.23;S,17.61;Found:C,39.77;H,1.84;N,19.44;S,17.72.

3.6.3.3,5-Dithioxo-7-(3,4,5-trimethoxy-phenyl)-2,3,5,6-tetrahydro[1,2,4]triazolo[4,3-c]pyrimidine-8-carbonitrile **6c**.Yield 55%, m.p.243-245˚C.IR (max/cm^{-1}):3345-3166 (NH),2200(CN),1652(C=S),1580(C=N).^{1}HNMR(300MHz,DMSO-d_6):δ3.1-3.7(s,9H, 3OCH_3),7,7.4(s,2H,Ar-H),9.1,10.2(s,2H,NH,D_2Oexchangeable).^{13}CNMR(300 MHz, DMSO-d_6):δ55.4,55.7,79.5,115.1,117.4,129.2,132.4,148.5,167,174,179.2,186.MS (EI): m/z: 375[M+] (37%).Anal.Calcd for $C_{15}H_{13}N_5O_3S_2$ (375.42): C,47.99; H,3.49; N,18.65; S,17.08; Found: C,48.21; H,3.60; N,18.78;S,17.15.

3.7.General method for preparation of 7a- i

A mixture of appropriate thiouracil derivative **1a - c**(0.01 mol) and appropriate aldehyde (0.01 mol) in ethanol (30 mL) was heated under reflux for 4h. The solid obtained was crystallized from benzene to yield compounds **7a- i**, respectively.

3.7.1.4-[(2E)-2-benzylidene-hydrazino]-6-(4-fluoro-phenyl)-2-thioxo-1,2,3,4-tetrahydr-opyrimidine-5-carbonitrile **7a**.Yield 70%, m.p.148-150 ˚C. IR(max/cm^{-1}): 3375-3180 (NH),2213(CN),1608(C=N).^{1}HNMR(300MHz,DMSO-d_6):δ2.3(s,1H,NH,D_2O exchang-eable),6.9-7.8(m,8H,Ar-H),8.6(s,1H,N=CH),9.9(s,1H,NH,D_2Oexchangeable).^{13}CNMR (300MHz,DMSO-d_6):δ88,115.5,116.2,127.4,127.8,129.4,130.5,132.5,151.1,154.1,161.3

,164,168.2,180.MS(EI):m/z:349[M+](25%).Anal.Calcd for $C_{18}H_{12}FN_5S$(349.38): C,61.88;H,3.46; N,20.04;S,9.18; Found:C,61.95;H,3.66;N,20.12;S,9.26.

3.7.2.4- [(2E)-2-benzylidene-hydrazino]-6-(4-bromo-phenyl)-2-thioxo-1,2,3,4-tetrahydr-opyrimidine-5-carbonitrile **7b**.Yield 72%,m.p.158-160˚C.IR(max/cm^{-1}):3380-3180(NH),2220(CN),1606(C=N).^{1}HNMR(300MHz,DMSO-d_6):δ2.2(s,1H,NH,D_2O exchangeable),7-7.8(m,8H,Ar-H),8.5(s,1H, N=CH),9.7(s,1H, NH,D_2O exchangeable).^{13}C NMR(300 MHz, DMSO-d_6): 86,115,116.2,124.4,127.1,127.4,129.4,133.3,143.7,151.1,155.2,164, 167.3,180.MS(EI):m/z:409[M+] (22.3%),411[M+2] (22.1%).Anal.Calcd for $C_{18}H_{12}BrN_5S$ (409):C,52.69; H,2.95; N,17.07;S,7.82;Found: C,52.79; ,3.04; N,17.16; S,7.95.

3.7.3.4- [(2E)-2-benzylidene-hydrazino]-6-(3,4,5-trimethoxy-phenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile **7c**.Yield 75%,m.p.123-125˚C.IR (max/cm^{-1}): 3369-3190(NH),2215CN),1605(C=N).^{1}HNMR(300MHz,DMSO-d_6):δ2.1(s,1H,NH,D_2Oexchangeable),3-3.7(s,9H,3OCH_3),7-7.9(m,6H,Ar-H),8.6(s,1H,N=CH),9.6(s,1H,NH,D_2O exchangeable).^{13}CNMR(300MHz,DMSO-d_6):δ55.5,55.7,85,115,117,122.3,127.4, 128.4,129.4,131.7,133.9,151.1,156.2,162,166.2,180.MS(EI):m/z:421[M+](20%).Anal. Calcd for $C_{21}H_{19}N_5O_3S$(421.47):C,59.84;H,4.54;N,16.62;S,7.61;Found:C,59.98;H,4.66; N,16.75; S,7.68.

3.7.4.4- [(2E)-2-(4-fluorobenzylidene)-hydrazino]-6-(4-fluoro-phenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile **7d**.Yield 72%, m.p.157-159˚C.IR(max/cm^{-1}): 3460-

3200(NH),2225(CN),1610(C=N).^{1}HNMR(300MHz,DMSO-d_6):δ2.4(s,1H,NH,D_2O exchangeable),6.8-7.8(m,8H,Ar-H),8.4(s,1H,N=CH),9.6(s,1H,NH,D_2Oexchangeable). MS(EI):m/z:367[M+](12%).Anal.Calcdfor$C_{18}H_{11}F_2N_5S$(367.37):C,58.85;H,3.02;N, 19.06;S,8.73;Found:C,58.98;H,3.14; N,19.20;S,8.88.

3.7.5.4- [(2E)-2-(4-fluorobenzylidene)- hydrazino]-6-(4-bromo-phenyl)-2-thioxo-1,2, 3, 4-tetrahydropyrimidine-5-carbonitrile **7e**.Yield73%,m.p.166-168˚C.IR(max/cm^{-1}): 3490 -32640(NH),2222(CN),1611(C=N).^{1}HNMR(300MHz,DMSO-d_6):δ2.3(s,1H,NH,D_2O exchangeable),7-7.8(m,8H,Ar-H),8.5(s,1H,N=CH),9.5(s,1H,NH,D_2O exchangeable).MS (EI):m/z:427[M+](10%).429[M+2](10.3).Anal.Calcd for $C_{18}H_{11}BrFN_5S$(426.99):C,50. 48;H,2.59;N,16.35;S,7.49; Found:C,50.57;H,2.66;N,16.55;S,7.54 .

3.7.6.4- [(2E)-2-(4-fluorobenzylidene)-hydrazino]-6-(3,4,5-trimethoxy-phenyl)-2-thioxo -1,2,3,4-tetrahydropyrimidine-5-carbonitrile **7f**.Yield 70%,m.p.128-130˚C.IR (max / cm^{-1}):3490-3300(NH),2220(CN),1609(C=N).^{1}HNMR(300MHz, DMSO-d_6): δ2.2 (s,1H, NH, D_2O exchangeable),3-3.7(s,9H,3OCH_3) ,6.7-7.8 (m,6H,Ar-H),8.5 (s,1H,N=CH),9.7 (s,1H,NH,D_2O exchangeable).MS(EI):m/z:439[M+](13%).Anal.Calcd for $C_{21}H_{18}FN_5O_3S$(439.46):C,57.39;H,4.13; N,15.94; S,7.30; Found: C,57.49; H,4.24; N,16.04; S.7.42.

3.7.7.4- [(2E)-2-(4-methoxybenzylidene)-hydrazino]-6-(4-fluoro-phenyl)-2-thioxo-1,2,3, 4-tetrahydropyrimidine-5-carbonitrile **7g**.Yield 72%,m.p.155-157˚C.IR(max/cm^{-1}): 3450-3200(NH), 2215(CN),1608 (C=N).^{1}H NMR (300 MHz, DMSO-d_6):δ 2.49 (s,1H, NH,D_2O exchangeable),3.37(s,3H,OCH_3),7.16-8.39(m,8H,Ar-H)8.59(s,1H,N=CH),9.5

(s,1H,NH,D_2O exchangeable).MS(EI):m/z:379[M+](14%).Anal.Calcd for $C_{19}H_{14}FN_5OS$ (379.41):C,60.15;H,3.72;N,18.46;S,8.45; Found: C,60.26;H,3.78;N,18.59; S,8.51.

3.7.8.4- [(2E)-2-(4-methoxybenzylidene)-hydrazino]-6-(4-bromo-phenyl)-2-thioxo-1,2,3, 4-tetrahydropyrimidine-5-carbonitrile **7h**.Yield 69%, m.p.152-154˚C.IR(max/cm^{-1}) : 3500-3200(NH),2217(CN),1607(C=N).^{1}H NMR(300 MHz, DMSO-d_6):δ2.3 (s,1H,NH, D_2O exchangeable),3.5(s,3H,OCH_3),7-7.8 (m,8H,Ar-H),8.5(s,1H,N=CH),9.5(s,1H,NH, D_2O exchangeable).MS (EI):m/z:439[M+](11.2%),441[M+2](11.4%).Anal. Calcd for $C_{19}H_{14}BrN_5OS$(439.01):C,51.83;H,3.20;N,15.91;S,7.28;Found:C,51.96; H,3.38;N,16. 08;S,7.34.

3.7.9.4- [(2E)-2-(4-methoxybenzylidene)-hydrazino]-6-(3,4,5-trimethoxy-phenyl)-2-thiox o-1,2,3,4-tetrahydropyrimidine-5-carbonitrile **7i**.Yield 68%, m.p. 134-136˚C.IR (max / cm^{-1}):3550-3300(NH),2225(CN),1609(C=N).^{1}HNMR(300 MHz,DMSO-d_6):δ2.2(1s,1H, NH,D_2O exchangeable),3-3.81 (s,12H,4OCH_3),7-7.9(6H,m,Ar-H),8.5(s,1H,N=CH),9.7 (s,1H,NH,D_2O exchangeable).MS(EI):m/z:451[M+](15%).Anal.Calcd for $C_{22}H_{21}N_5O_4S$ (451.49):C,58.52;H,4.69;N,15.51;S,7.10;Found:C,58.70;H,4.78;N,15.80;S,7.15.

3.8. General method for preparation of 8a- c

A solution of appropriate thiouracil derivative **1a - c** (0.01 mol) and diethyloxalate (0.01 mol) in absolute ethanol (40 mL) was heated under reflux for 10 h. The solid obtained was crystallized from benzene to yield compounds **8a- c**,respectively.

3.8.1.8-(4-Fluoro-phenyl)-3,4-dioxo-6-thioxo-3,4,6,7-tetrahydro-2H-pyrimido[6,1-c]- [1,2, 4]triazine-9-carbonitrile **8a**.Yield 65%, m.p.228-230˚C. IR (max/cm^{-1}):3400-3220

(NH),2230(CN),1664(C=O),1275(C=S).^{1}HNMR(300MHz,DMSO-d_6):δ6.59-7.07(m,4H ,Ar-H),10,11.7(s,2H,NH,D_2O exchangeable).^{13}CNMR(300MHz,DMSO-d_6): δ82.5,113, 117.3,124.4,127.1,143.7,155.2,161.7,165.9,176.7,178.5.MS(EI):m/z:315[M+](8%).Anal .Calcd for $C_{13}H_6FN_5O_2S$(315.28):C,49.52;H,1.92;N,22.21;S,10.17; Found:C,49.66;H,2. 02;N,22.45;S,10.26.

3.8.2.8-(4-Bromo-phenyl)-3,4-dioxo-6-thioxo-3,4,6,7-tetrahydro-2H-pyrimido[6,1-c]- [1,2,4]triazine-9-carbonitrile **8b**.Yield 66%,m.p.220-222˚C.IR (max/cm^{-1}):3455-3200 (NH),2213(CN),1671(C=O),1270(C=S).^{1}HNMR(300MHz,DMSO-d_6):δ7-7.5(m, 4H,Ar- H),8.2,9.3(s,2H,NH,D_2O exchangeable).^{13}CNMR(300MHz,DMSO-d_6):δ85.6,117.1,122. 3,128.4,131.7,133.9,157.3,163,166.2,175.7,178.6.MS(EI):m/z:375[M+] (10.2%),377[M +2](10.4%).Anal.Calcd for $C_{13}H_6BrN_5O_2S$(374.94):C,41.51;H,1.61;N,18.62;S, 8.52; Found:C,41.75; H,1.77;N,18.79;S,8.66.

3.8.3.3,4-Dioxo-6-thioxo-8(3,4,5-trimethoxy-phenyl)-3,4,6,7-tetrahydro-2H-pyrimido [6,1-c]- [1,2, 4]triazine-9-carbonitrile **8c**.Yield 69%, m.p.250-252˚C. IR (max/cm^{-1}): 3500-3210(NH),2215(CN),1677(C=O),1275(C=S).^{1}HNMR(300MHz,DMSO-d_6):δ3- 3.74(s,9H,3OCH_3,7,7.4(s,2H,Ar-H),8.5,9(s,2H,NH,D_2Oexchangeable).^{13}CNMR(300 MHz,DMSO-d_6):δ,55.4,55.7,80.7,115.1,117.2,129.2,132.4,148.5,155.3,161.3, 165.9, 175.2,176.1.MS(EI):m/z:387[M+](7%).Anal.Calcd for $C_{16}H_{13}N_5O_5S$(387.36): C,49.61; H,3.38; N,18.08;S,8.28;Found: C,49.80; H,3.45; N,18.28;S,8.34.

3.9. General method for preparation of 9a- c

A mixture of appropriate thiouracil derivative **1a - c** (0.01mol) and triethyl orthoformate (30 mL) was heated under reflux for 2 h. The solid obtained was crystallized from acetic acid to yield compounds **9a- c**, respectively.

3.9.1.7-(4-Fluoro-phenyl)-5-thioxo-5,6-dihydro[1,2,4] triazolo[4,3-c]-pyrimidine-8-carbonitrile **9a**.Yield 61%, m.p.165-167˚C.IR (max/cm^{-1}):3251(NH),2218(CN),1580(C=N) .^{1}H NMR (300MHz,DMSO-d_6): δ7.5-7.55 (m, 4H, Ar-H),8.1(s,1H,C_5-H),10.47 (s,1H, NH,D_2O exchangeable).^{13}C NMR (300 MHz, DMSO-d_6): 85.4, 112,117.2,122.3,131.7, 142.2,148,155,168.3,184.MS (EI):m/z:271[M+](40%).Anal.Calcd for $C_{12}H_6FN_5S$ (271. 27):C,53.13;H,2.23;N,25.82;S,11.82; Found:C,53.25;H,2.33;N,25.95; S,11.90.

3.9.2.7-(4-Bromo-phenyl)-5-thioxo-5,6-dihydro[1,2,4]triazolo[4,3-c]-pyrimidine-8-carbonitrile **9b**.Yield 62%,m.p.180-182˚C.IR(max/cm^{-1}):3218(NH),2217(CN),1585(C=N). ^{1}HNMR(300MHz,DMSO-d_6):δ7-7.6 (m,4H, Ar-H),8.60 (s,1H,C_5-H),9.5(s,1H, NH,D_2O exchangeable).^{13}CNMR(300MHz,DMSO-d_6):85.5,112,117.9, 129.2,131.3,148.5,155.6, 160.2,167.6,182. MS (EI): m/z: 331 [M+] (30.3%).333 [M+2] (30.4%). Anal.Calcd for $C_{12}H_6BrN_5S$(330.95):C,43.39; H,1.82; N,21.08; S,9.65; Found: C, 43.48; H, 1.96; N, 21.19; S, 9.76.

3.9.3.5-Thioxo-7-(3, 4,5-trimethoxy-phenyl)-5,6-dihydr [1,2,4] triazolo [4,3-c]-pyrimidine-8-carbonitrile **9c**.Yield 65%,m.p.188-190˚C.IR (max/cm^{-1}):3230(NH), 2219(CN), 1586(C=N).^{1}H NMR(300 MHz, DMSO-d_6):δ3-3.7(s,9H,3OCH_3),7.2,7.7(s,2H,Ar-H), 8.62(s,1H,C_5-H),9.3(s,1H,NH ,D_2O exchangeable).^{13}CNMR (300MHz,DMSO-d_6): 55.4,55.6,90,115,117.9,127.8,130.5,148.3,155,158,166,178. MS(EI): m/z: 343 [M+] (51%). Anal.Calcd for $C_{15}H_{13}N_5O_3S$ (343.36): C,52.47; H,3.82; N,20.40; S,9.34;

Found: C,52.55;H,3.99;N,20.65;S,9.44 .

3.10. General method for preparation of 10 a- c

A mixture of appropriate thiouracil derivative **1a- c** (0.01 mol) and acetyl acetone (0.1 mL, 0.001 mol) in acetic acid (15 mL) was refluxed for 15h. The reaction mixture was cooled and poured onto ice - water. The solid obtained was crystallized from ethanol to yield compounds **10a- c**,respectively.

3.10.1.4-(3,5-dimethyl-1H-pyrazol-1-yl)-6-(4-fluoro-phenyl)-2-thioxo-1,2-dihydropyrim-idine-5-carbonitrile **10a**.Yield 50%,m.p.245-247˚C.IR(max/cm^{-1}):3325(NH),2220 (CN), 1606 (C=N).^{1}H NMR (300 MHz, DMSO-d_6):δ2,2.5(s, 6H, $2CH_3$),6.7(s,1H ,C_4-H),7- 7.5 (m,4H,Ar-H),9.1(s,1H,NH,D_2Oexchangeable).^{13}CNMR(300MHz,DMSO-d_6):11.78, 14. 04,86.78,111.64,113.89,115.28,126.89,127.92,130.04,147.32,152.47,158.51,159.66,175. 72.MS(EI):m/z:325[M+](23%),Anal.Calcd for $C_{16}H_{12}FN_5S$(325.36):C,59.06;H,3.72 ; N,21.52; S,9.86; Found: C,59.18; H,3.90; N, 21.77; S,9.92.

3.10.2.4-(3, 5-dimethyl-1H-pyrazol-1-yl)-6-(4-bromo-phenyl)-2-thioxo-1,2-dihydro pyrimidine-5-carbonitrile **10b**.Yield 52%,m.p.238-240˚C.IR(max/cm^{-1}):3345(NH),2225 (CN),1608(C=N).^{1}HNMR(300MHz,DMSO-d_6):δ2.25,2.4(s,6H,$2CH_3$),6.8(s,1H,C_4-H), 7.2-7.7(m,4H,Ar-H),9.3(s,1H,NH,D_2Oexchangeable).^{13}CNMR(300MHz,DMSO-d_6):12, 15.3,90,110,115,117,129.2,132.4,148,149,152,162,169,179.MS(EI):m/z:385[M+](30.1 %).387 [M+2] (30.3%). Anal. Calcd for $C_{16}H_{12}BrFN_5S$ (385):C,49.75; H,3.13; N,18.13; S,8.30; Found: C,49.95; H,3.41; N,18.29;S,8.39.

3.10.3.4-(3, 5-Dimethyl-1H-pyrazol-1-yl)-2-thioxo-6(3,4,5-trimethoxy-phenyl)-1,2-di-hydropyrimidine-5-carbonitrile **10c**.Yield 53%, m.p. 230-232˚C. IR (max/cm^{-1}):3365: (NH),2222(CN),1609(C=N).^{1}HNMR(300MHz,DMSO-d_6):δ2.4,2.53(s,6H,2CH_3),3-3.7 (s,9H,3OCH_3),6.73(s,1H,C_4-H),7.2,7.3(s,2H, Ar-H),9.3(s,1H,NH,D_2O exchangeable). ^{13}CNMR(300 MHz,DMSO-d_6):11,19.3,55.4,55.7,92,111,114,117, 127.2 ,134.4,146, 147,155,163,165,177.MS(EI):m/z:397[M+](32%).Anal.Calcd for $C_{19}H_{19}N_5O_3S$ (397. 45):C,57.42;H,4.82;N,17.62; S,8.07;Found:C,57.66;H,4.95;N, 7.77;S,8.27.

3.11.General method for preparation of 11a- c

A cold solution (0 - 5˚C) of sodium nitrite (1g, 0.144mol) in H_2O (15 mL) was added gradually within 15 min to a cold stirred solution of appropriate thiouracil derivative **1 a- c** (0.01 mol) in 2N HCl (15mL).After addition, the reaction mixture was then further stirred for 4 h at the same temperature ,then it was diluted with cold H_2O. The solid obtained was crystallized from ethanol to yield compounds **11a- c**, respectively.

3.11.1.7-(4-Fluoro-phenyl)-5-thioxo-5,6-dihydrotetrazolo[1,5-c]pyrimidine-8-carbonit-rile **11a**.Yield 50%, m.p.218-220˚C.IR (max/cm^{-1}):3325(NH),2220 (CN),1510 (C=N). ^{1}HNMR(300MHz,DMSO-d_6):δ7-7.6(m,4H,Ar-H),9(s,1H,NH,D_2O exchangeable).MS (EI):m/z:272[M+](45%).Anal.Calcd for $C_{11}H_5FN_6S$(272.26):C,48.53;H,1.85; N,30.87 ;S,11.78;Found:C,48.67;H,1.98;N,30.95;S,11.88.

3.11.2.7-(4-Bromo-phenyl)-5-thioxo-5,6-dihydrotetrazolo[1,5-c]pyrimidine-8-carbonit-

rile **11b**.Yield 54%, m.p. 208-210˚C.IR (max/cm^{-1}):3330(NH),2223 (CN),1521 (C=N). ^{1}H NMR (300MHz,DMSO-d_6):δ7.1-7.8 (m,4H,Ar-H),9.4(s,1H,NH, D_2O exchangeable). MS(EI):m/z:332[M+](60.1%).334 [M+2](60.3%).Anal. Calcd for $C_{11}H_5BrN_6S$ (331.95): C,39.66; H,1.51;N,25.22;S,9.62; Found:C,39.79;H,1.72;N,25.45; S,9.80.

5-Thioxo-7-(3,4,5-trimethoxy-phenyl)-5,6-dihydrotetrazolo[1,5-c]pyrimidine-8-carbonitrile **11c**.Yield 55 %,m.p.200-202˚C.IR (max/cm^{-1}):3322(NH),2222 (CN), 1520 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6):δ3.4-3.8(s,9H,3OCH_3),6.6,6.8(s,2H,Ar-H),10.14(s,1H ,NH,D_2O exchangeable).MS(EI):m/z:344[M+](56%).Anal.Calcd for $C_{14}H_{12}N_6O_3S$ (344. 34): C,48.83; H,3.51; N,24.41; S,9.31; Found:C,48.96; H,3.76;N, 24.66; S,9.42.

Biological assay

Antimicrobial activity

Materials and methods

Antibacterial activity was examined by the disc-diffusion method and the MIC method under standard conditions of the National Committee for Clinical Laboratory Standards [37]. The in-vitro antimicrobial activity of the synthesized compounds was investigated against several pathogenic representative Gram-positive bacteria (*S.aureus* ATCC12600 , *B.cereus* ATCC14579), Gram-negative bacteria (*Escherichia coli* ATCC11775), and (*Candida albicans* ATCC26555,*Aspergillus flavus* ATCC 11495) as a representative for fungi. All microorganisms used were obtained from culture collection of the Department of Microbiology, Micro Analytical Centre, Faculty of Science, Cairo University, Cairo, Egypt. Media for disc sensitivity tests were the nutrient agar and Muller-Hinton agar (MHA) purchased from Difco (USA). Non-sterile powder of the tested compound was dissolved in sterile DMSO to yield: 10 m*g* mL^{-1}, and passed through a 0.2μm membrane

filter (Millipore Corp, USA). The filtrates were dispensed as 2 mL samples into sterile, small screw-capped vials, frozen and kept stored at ⁻15 °C. The vials were refrozen after thawing. Disc diffusion sensitivity test was done in the manner identical to that of Bauer et al. [38]. DMSO showed no inhibition zones.

Penicillin (Bioanalyse, Turkey) and Fluconazole (Sigma- Aldrich, USA) were used as reference substances. Inhibition zones were measured in millimeters at the end of an incubation period of 48 h at 28 °C (see Inline Supplementary,Figs.S1-S10).

MIC (minimum inhibitory concentration) for each tested compound was determined on Mueller⁻Hinton agar (MHA), by micro dilution technique according to NCCLS guidelines 1997 [39].All bacterial isolates were sub cultured in MHA plates and incubated overnight at 37 °C and all Candida isolates were sub cultured in SDA plates at 35 °C for 24-48 h. The microorganisms were passage at least twice to ensure purity and viability. The solution of the newly synthesized compounds and standard drugs were prepared at 1024,512,256,128,64,32 and 16 μg/mL concentrations using serial two folds dilutions in DMSO (dimethyl sulphoxide), each concentration was mixed with sterile nutrient agar (Sigma - Aldrich) in sterile plate, bacteria inoculums were added to each well of the micro dilution trays. The trays were incubated at 37°C in a humid chamber and MIC end points were read after 24 h of incubation.

Antioxidant activity

Determination of radical scavenging activity using DPPH assay:

Thiouracil derivatives (**1**, **2**, **5a**, **c),7a**,**7g** and **8a**, **c** were evaluated for their antioxidative potential through invitro DPPH radical scavenging model. The DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging effect was carried out according to reported methods

[40,41]. Compounds of different concentrations were prepared in DMSO, 1 mL of each compound solutions having different concentrations (10, 25,50,100,200 and 300 μM) were taken in different test tubes, 4 mL of 0.1 mM DMSO solution of DPPH was added and shaken vigorously. The tubes were then incubated in the dark room at room temperature for 20 minutes. A DPPH blank was prepared without compound, and DMSO was used for the baseline correction. Changes (decrease) in the absorbance at 517 nm were measured using a UV-visible spectrophotometer (Shimadzu 160A). Scavenging of DPPH free radicals was calculated from the following equation:

DPPH scavenging activity (%) = [(Ac-At)/Ac] × 100

Where; Ac is absorbance of control, At is absorbance of compound.

Results

The relation between DPPH scavenging percentage against compound concentrations is plotted to get compound concentration providing a 50% decrease in absorbance of DPPH radical (IC_{50}%). The radical scavenging activities were expressed as IC_{50}. Vitamin C served as reference compound. The results have been given in (Table II , Fig.4, 5).

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Figures and Schemes captions.

Fig. 1 .Structures of some potent 5-cyano -2-thiouracils.
Fig. 2: Antibacterial activity (Gram +ve,-ve) of synthesized compounds.
Fig. 3: Antifungal activity of synthesized compounds.
Fig. 4. Screening of antioxidant activity by the DPPH assay shows that 1c, 5c and 8c have highest activity (more potent than the reference drug (RF). Each value represents a mean ± SEM (n= 3).

Fig.5. DPPH radical scavenging activity of compound 1c. Each value represents a mean ± SEM (n = 3).

Scheme 1. Synthetic Pathway for the preparation of compounds 1 ~ 6 (a - c).

Scheme 2. Synthetic Pathway for the preparation of compounds 7 ~11 (a - c).

Table I. Antimicrobial activity results of newly synthesized compounds

, expressed as inhibition zone diameter (mm) and MIC (μg/ml) with the standard drugs.

Compds & Stander	Inhibition zone diameter (mm) and MIC of tested compounds (μg/ml)				
	S.aureus ATCC 12600	*B.cereus* A TCC 14579	*E.coli* ATCC 11775	*C.albicans* A TCC 26555	*Asp.flavus* ATCC 11495
1a	15(16)	12(64)	14(32)	NT	13(32)
1b	13(64)	11(64)	11(64)	NT	NT
1c	11(64)	10(64)	12(32)	NT	NT
3a	7(128)	6(128)	8(128)	8(128)	7(256)
3b	6(128)	5(128)	7(128)	8(128)	7(256)
3c	7(128)	5(128)	6(128)	8(128)	7(256)
4a	7(128)	5(128)	7(128)	8(128)	7(256)
4b	7(128)	6(128)	6(128)	8(128)	7(256)
4c	6(128)	6(128)	7(128)	8(128)	7(256)
5a	12(32)	NT	NT	NT	NT
5b	11(64)	NT	NT	NT	NT
5c	10(64)	NT	NT	NT	NT
6a	NT	11(64)	11(64)	12(32)	NT
6b	NT	10(64)	11(64)	12(32)	NT
6c	NT	11(64)	10(64)	12(32)	NT
7a	24(16)	22(16)	19(16)	19(16)	19(16)
7b	20(32)	17(32)	13(32)	16(16)	15(32)
7c	17(16)	18(16)	12(16)	15(16)	15(16)
7d	19(32)	16(32)	12(32)	15(32)	14(32)
7e	20(32)	16(32)	13(32)	15(32)	14(32)
7f	19(32)	17(32)	12(32)	16(32)	15(32)
7g	23(16)	21(16)	19(16)	18(16)	18(16)
7h	18(32)	16(32)	12(32)	16(32)	14(32)
7i	17(32)	15(32)	12(32)	15(32)	15(32)
Penicillin [a]	16(16)	13(32)	12(32)	NT	NT
Fluconazole[b]	NT	NT	NT	20(16)	16(16)

All tested compound = 10 mg /disc in DMSO, DMSO shows no activity.

a (Penicillin) =10 m*g*/ mL b(Fluconazole)= 50 µg /mL NT=Not tested , No inhibition

N.B: **2 a**,**b** and **c** were inactive toward all tested organisms in both used concentration.

Table I. Antimicrobial activity results of newly synthesized compounds

, expressed as inhibition zone diameter (mm) and MIC (μg/ml) with the standard drugs.

Compds & Stander	Inhibition zone diameter (mm) and MIC of tested compounds (μg/ml))				
	S.aureus ATCC 12600	*B.cereus* A TCC 14579	*E.coli* ATCC 11775	*C.albicans* A TCC 26555	*Asp.flavus* ATCC 11495
8a	14(32)	NT	NT	NT	18(16)
8b	13(32)	NT	NT	NT	17(16)
8c	14(32)	NT	NT	NT	17(16)
9a	22(16)	19(16)	14(16)	18(16)	19(16)
9b	21(16)	17(16)	12(16)	16(16)	17(16)
9c	21(16)	18(16)	13(16)	17(16)	18(16)
10a	13(32)	12(32)	11(32)	NT	NT
10b	12(32)	12(32)	11(32)	NT	NT
10c	11(32)	11(32)	12(32)	NT	NT
11a	15(16)	15(32)	13(32)	NT	NT
11b	14(16)	13(32)	12(32)	NT	NT
11c	14(16)	12(32)	12(32)	NT	NT
Penicillin[a]	16(16)	13(32)	12(32)	NT	NT
Fluconazole[b]	NT	NT	NT	20(16)	21(16)

All tested compound = 10 mg /disc in DMSO, DMSO shows no activity.

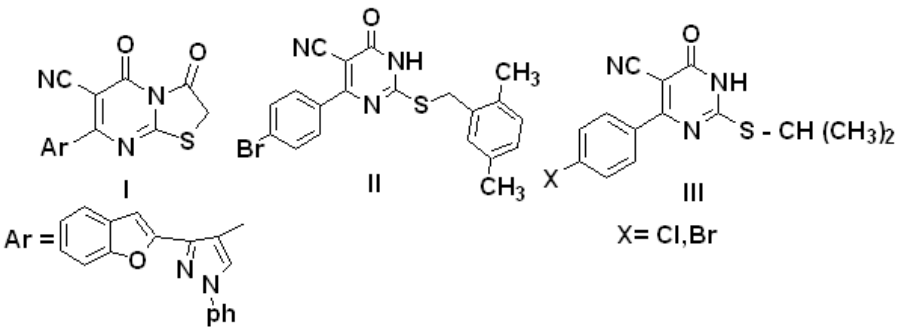
a (Penicillin) =10 m*g*/ mL b (Fluconazole)= 50 µg /mL NT=Not tested , No inhibition

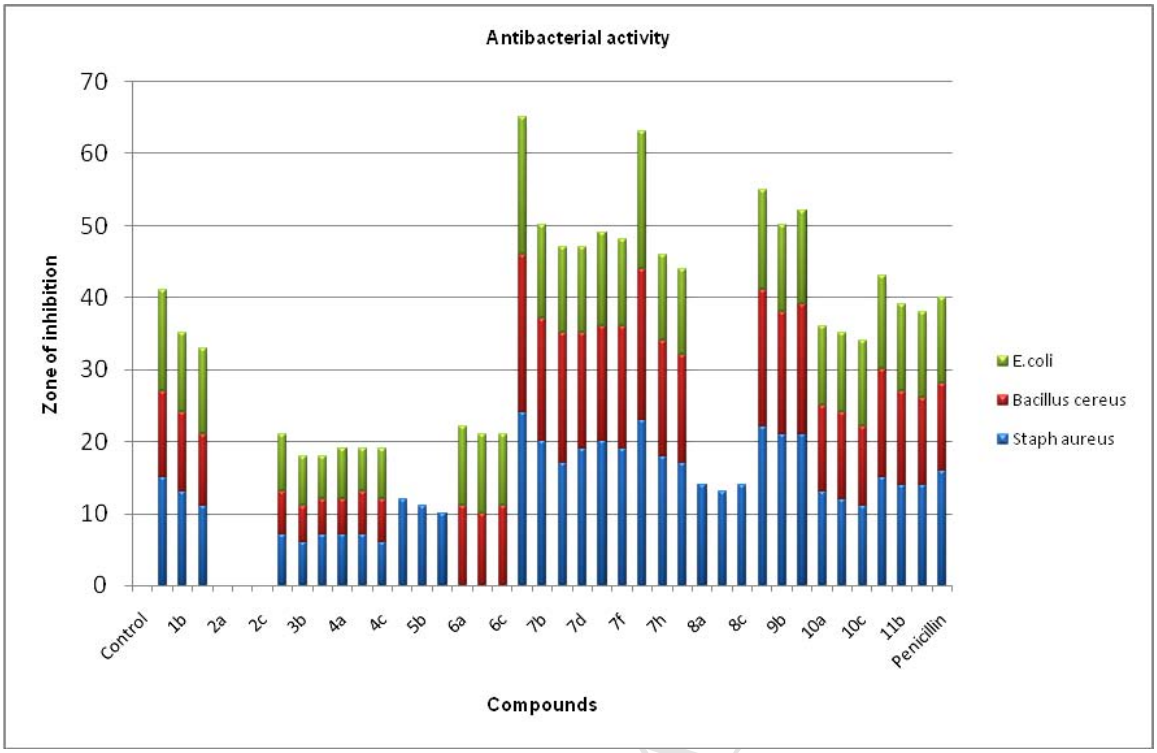
N.B: **2 a,b** and **c** were inactive toward all tested organisms in both used concentration.

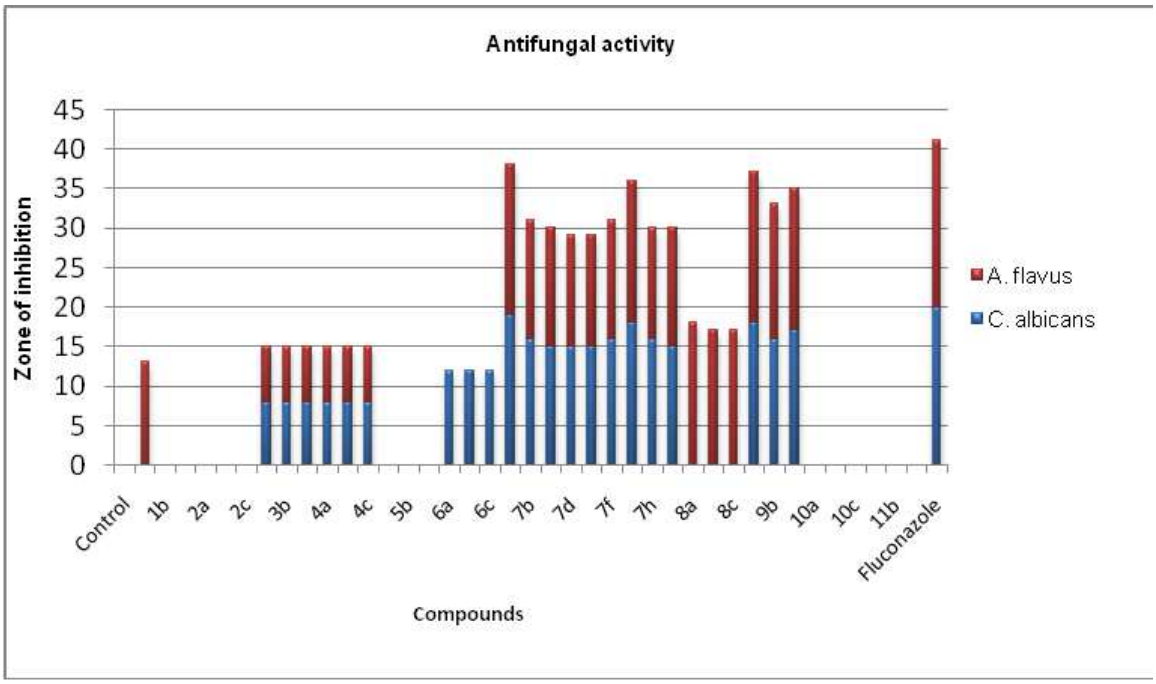
Table II. IC_{50} values [a] (in **µg/mL**) for DPPH scavenging ability of the compounds

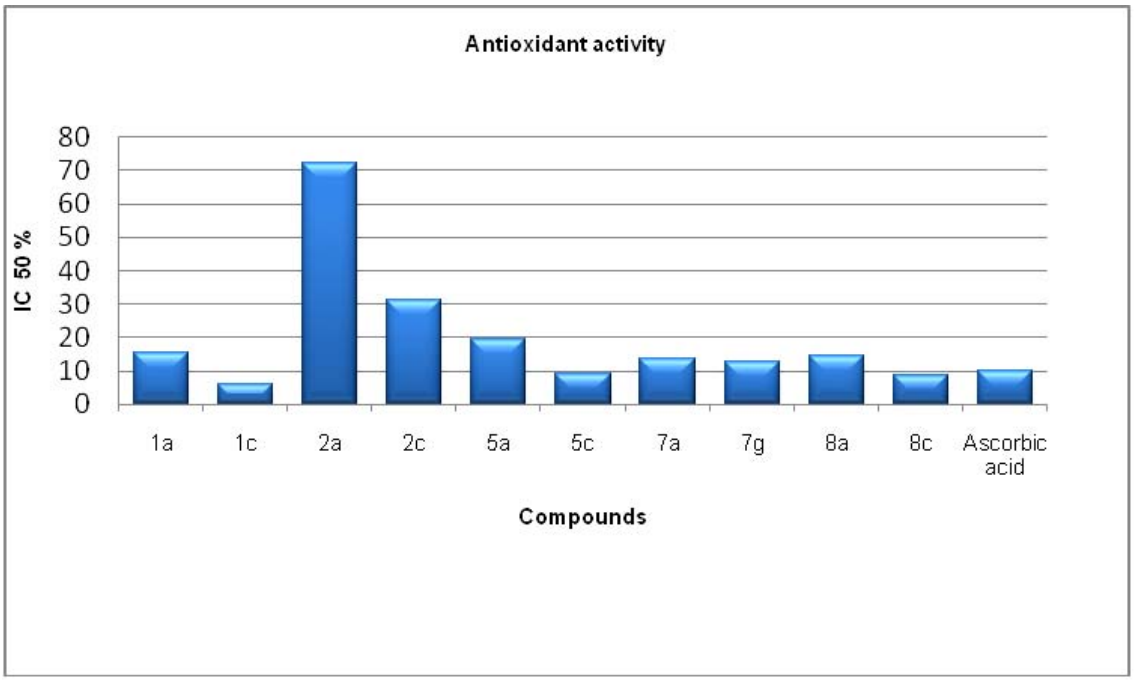
Compounds	**IC_{50} (µg/mL)**
1a	15.24 ± 0.012
1c	6.21 ± 0.014
2a	72.03 ± 0.021
2c	31.15 ± 0.015
5a	19.62 ± 0.031
5c	9.34 ± 0.02
7a	13.80 ± 0.01
7g	12.68 ± 0.01
8a	14.72 ± 0.032
8c	8.55 ± 0.042
Ascorbic acid	10 ± 0.043

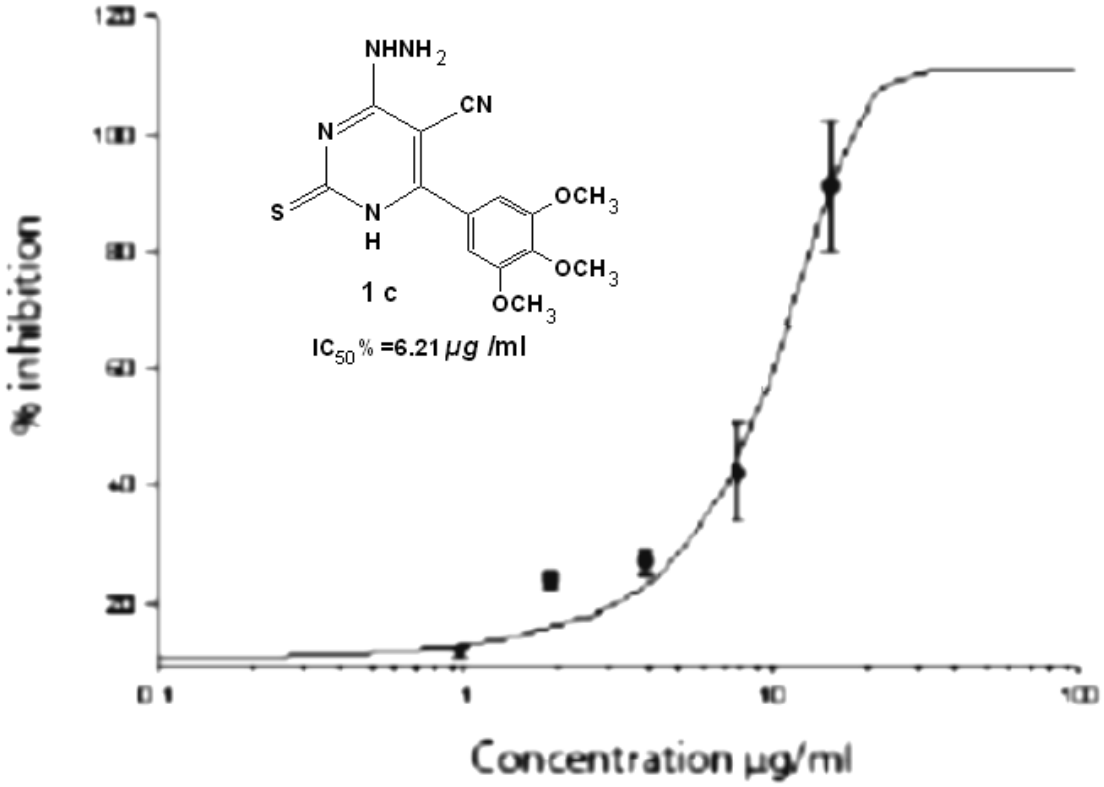
IC_{50} values [a] (in µg/mL) ,which the concentration required for a 50% decrease in absorbance of DPPH radical. Results are presented as a mean ± SEM (n=3), 8 replica each.

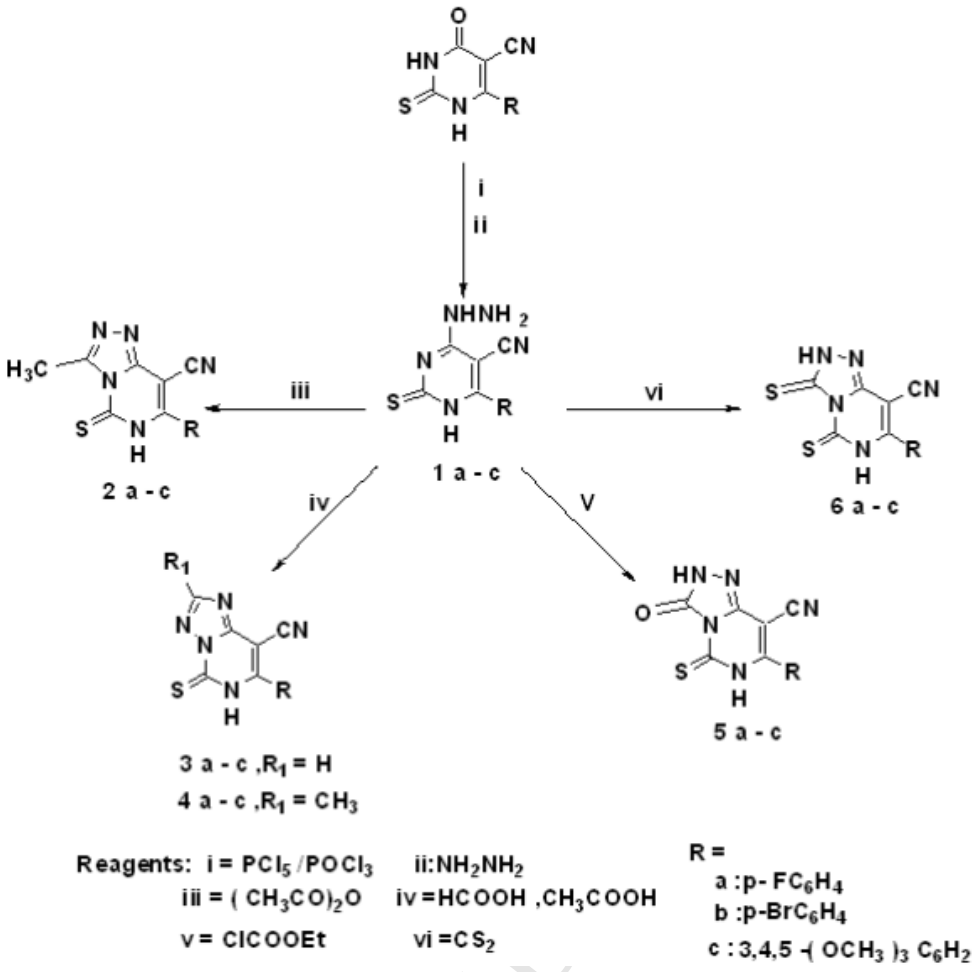


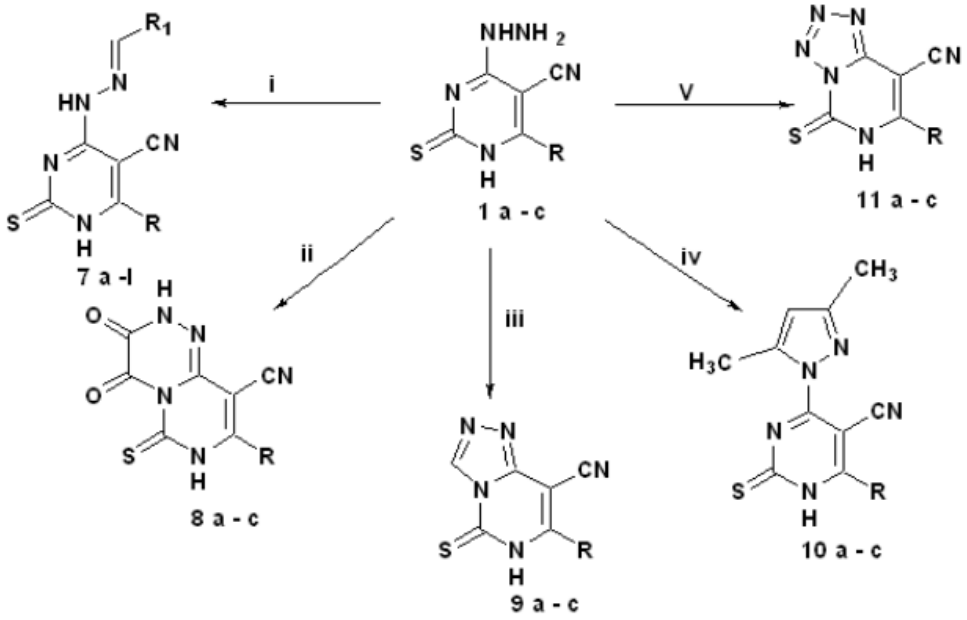









i
ii
iii
iv
V
vi
1 a - c
2 a - c
3 a - c ,R1 = H
4 a - c ,R1 = CH3
5 a - c
6 a - c
Reagents: i = PCl5 /POCl3 ii:NH2NH2
iii = (CH3CO)2O iv =HCOOH ,CH3COOH
v = ClCOOEt vi =CS2
R =
a :p- FC6H4
b :p-BrC6H4
c : 3,4,5 -(OCH3)3 C6H2



Reagents: i = PhCHO,EtOH ii =$(COOC_2H_5)_2$

iii = $HC(OC_2H_5)_3$ iv =$(CH_3CO)_2CH_2$

v =$NaNO_2$ /HCl

R =

a : p- FC_6H_4

b : p-BrC_6H_4

c : 3,4,5 -(OCH_3)$_3$ C_6H_2

R_1 =

C_6H_5 ,

P-FC_6H_5 ,

o-OCH_3-C_6H_4 .

Supplementary material

Some examples for ihhibition zones of tested compounds:

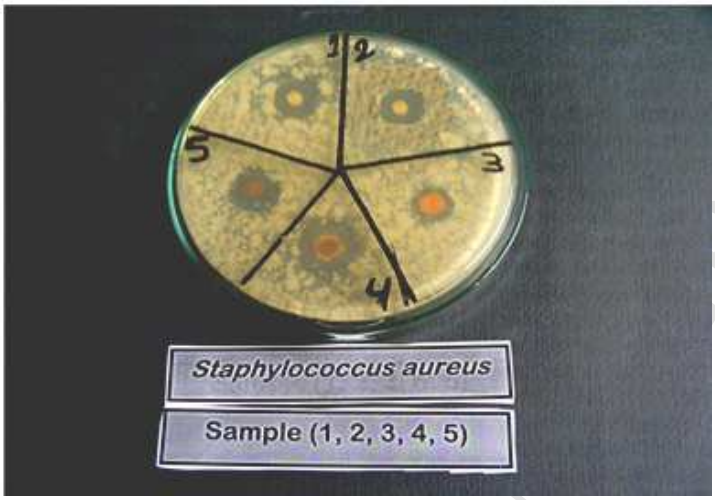


Fig. S1: Zone of inhibition of compounds: 7e, 7b, 7d, 7a and 7g in ascending sequence against S.aureus.

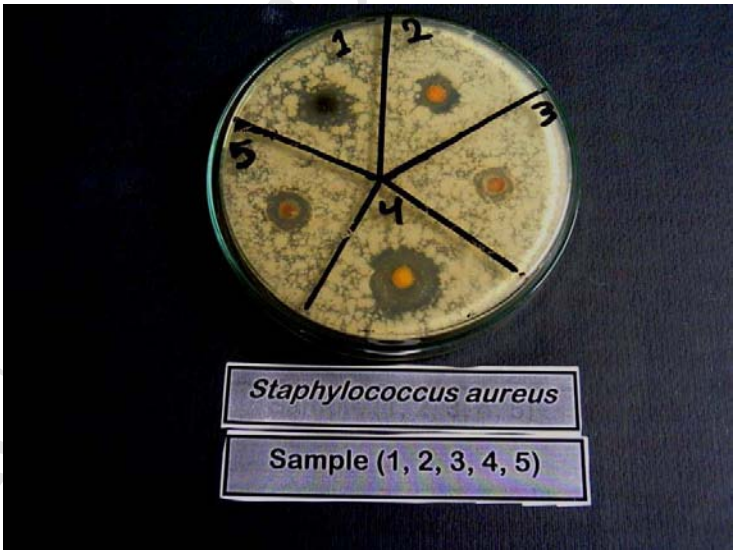


Fig. S2: Zone of inhibition of compounds :1b, 8a, 8b, 1a and 8c in ascending sequence against S.aureus.

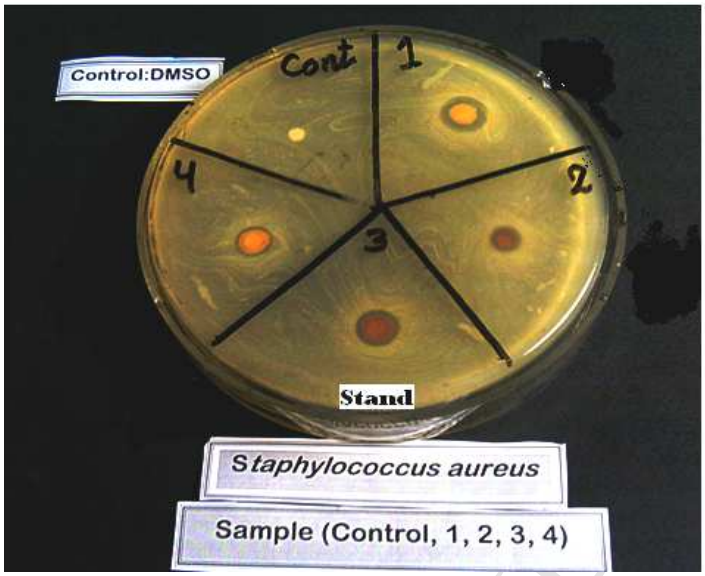


Fig. S3: Zone of inhibition of compounds: 9b, 11b, standard and 11a in ascending sequence against S.aureus.

□

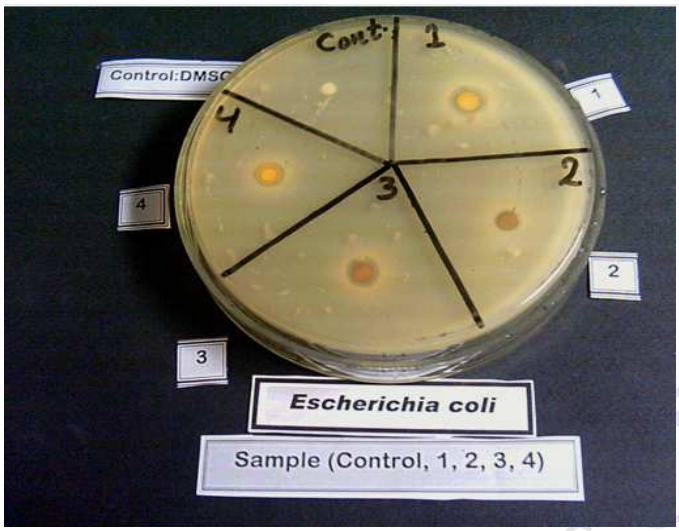


Fig. S4: Zone of inhibition of compounds: 7g, 7b, 7c and 6a in ascending sequence against E.coli.

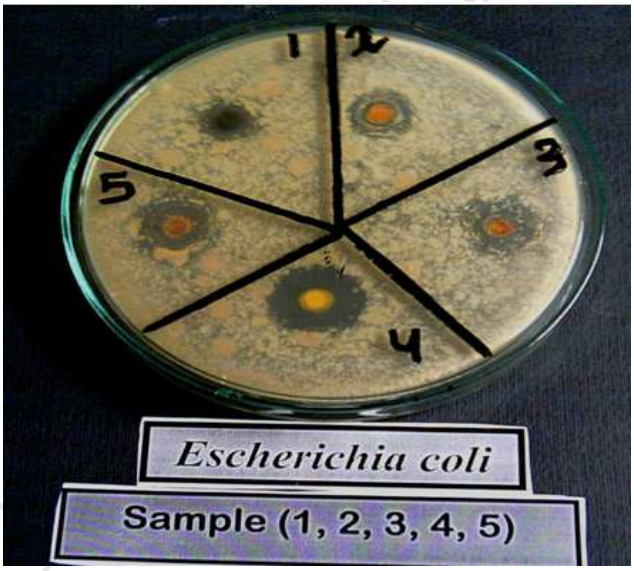


Fig. S5: Zone of inhibition of compounds: 11a, 9a, 1c, 7a and 1a in ascending sequence against E.coli.

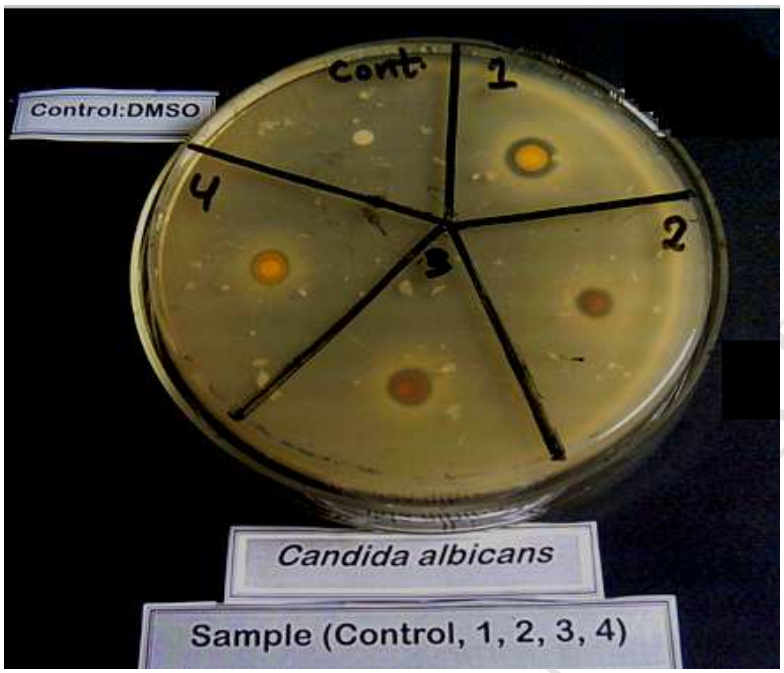


Fig. S6: Zone of inhibition of compounds: 7a, 7d, 7c and 7a in ascending sequence against C.albicans.

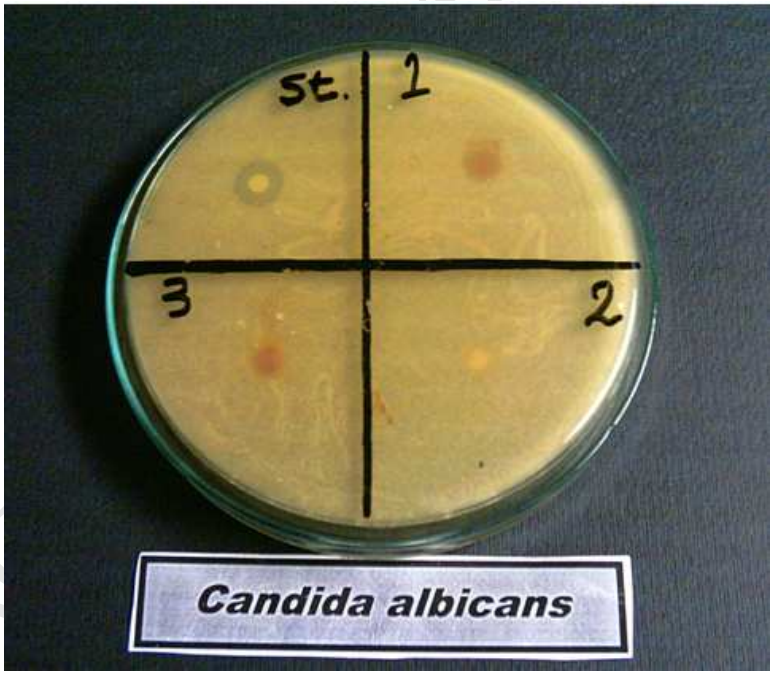


Fig.S7: Zone of inhibition of Compounds: standarad, 6a, 1a and 5a in ascending sequence against C.albicans.

Fig. S7: Zone of inhibition of compound 7a against Asp.flavus.

□

Fig.S8: Zone of inhibition of compounds: 9b,5a, 6b and10a in ascending sequence against Asp.flavus.

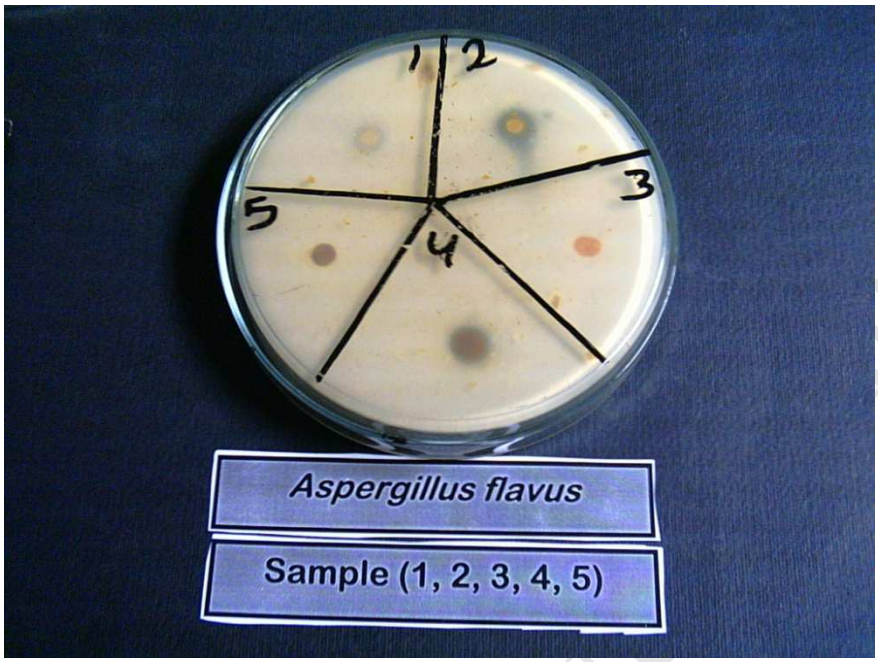


Fig.S9: Zone of inhibition of compounds: 1a,7b,1b,7e and 1c in ascending sequence against Asp.flavus.

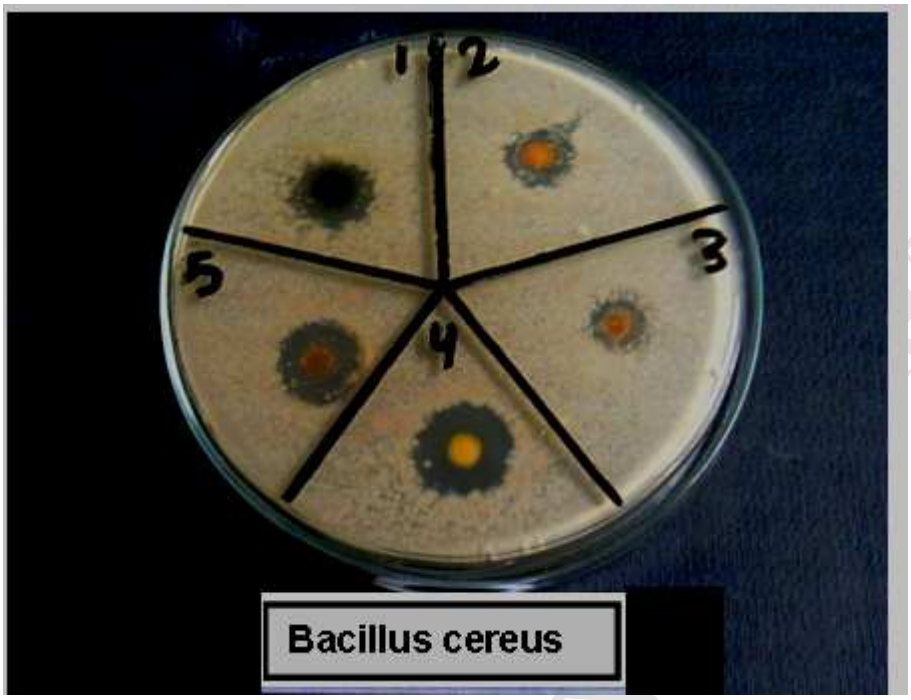


Fig. S10: Zone of inhibition of compounds 7g, 9c, 11a, 7a and 9a in ascending sequence against B.cereus.