Scientific paper

A Convenient Methods for Synthetic Isomeric Structures of Pyrimido-1,2,4-triazine Derivatives as Biocidal Agents

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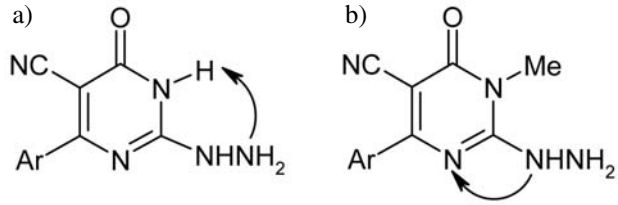
Abstract

Some new isomeric structures of pyrimido[2,1-*c*][1,2,4]triazines **4**–**8** and **10**–**14** have been synthesized via the ring closure reactions of 2-hydrazinyl-1-methylpyrimidine **3** and/or 2-hydrazinopyrimidine **9** with acyclic and cyclic oxygen compounds under various conditions. Structures of the targets have been established from their elemental analyses and spectral data (UV, IR, $^{1}H/^{13}C$ NMR and mass spectrometry). Most of the obtained compounds were evaluated as antimicrobial agents and compared with pipericillin and mycostatine as standard antibiotics. Only compound **7** had highly biocidal effects.

Keywords: Synthesis, isomeric structures, pyrimidotriazines, biocidal effects.

1. Introduction

Polyfunctional pyrimidines are highly reactive intermediates for building various heterobicyclic nitrogen systems which exhibit a broad spectrum of biological and pharmacological properties.[1–3] Diverse pharmacological properties of pyrimidine derivatives, such as anticancer,[4–6] antiinflammatory,[7,8] antimalarial,[9] antiviral,[10] and antidepressant,[11] and fused pyrimidines as antimicrobial,[12–14] antibacterial,[15] antifungal,[16] and antihypertensive[17] support the importance of their synthesis. On the other hand, 1,2,4-triazines have been proved to be very useful in the synthetic chemistry, especially in various one-step heterocyclization reactions proceeding by insertion of two carbon atoms bearing bifunctional groups.[18–20] The structural diversity and biological significance of 1,2,4-triazines have aroused much attention due to the wide range of applications.[21–25] In view of all these facts and as the continuation of our work on the synthesis of new heterocyclic derivatives,[26] the main aim of the present work is the study of the reactivity of polyfunctional pyrimidines with the aim of constructing fused heterobicyclic nitrogen systems containing 1,2,4-triazine moiety starting from 2-hydrazinopyrimidine via two routes (**A** and **B**), in view of the biocidal effects of the final products.



The isomeric structure targets

2. Results and Discussion

The original objective of this work is the formation of isomeric fused pyrimidotriazines via nitrogen atoms. Thus, starting with methylation of 4-(2-hydroxy-1-naphthyl)-2-mercapto-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (**1**),[27] using methyl iodide in stirring with aqueous KOH for one day, yielded 4-(2-hydroxy-1-naphthyl)-1-methyl-2-(methylthio)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (**2**) which on hydrazinolysis by refluxing with hydrazine hydrate in ethanol produced the corresponding 2-hydrazinyl-1-methylpyrimidine **3** (Scheme 1). Compound **3** was used as starting material for building of pyrimido[2,1-*c*][1,2,4]triazine via route **B**. UV absorption

spectrum of **2** showed λ_{max} 434 nm, while that of **3** exhibited λ_{max} 477 nm. The presence of hydrazino group in compound **3** shifted λ_{max} to longer wave length more than methylthio group in compound **2**.

When 2-hydrazinyl-1-methylpyrimidine **3** was subjected to react with monochloroacetic acid and/or chloroacetyl chloride in warm DMF the isomeric structures 4,8-dioxo-pyrimido[2,1-*c*][1,2,4]triazine **4** and 3,8-dioxopyrimido[2,1-*c*][1,2,4]triazine **5**, respectively, were formed (Scheme 1).

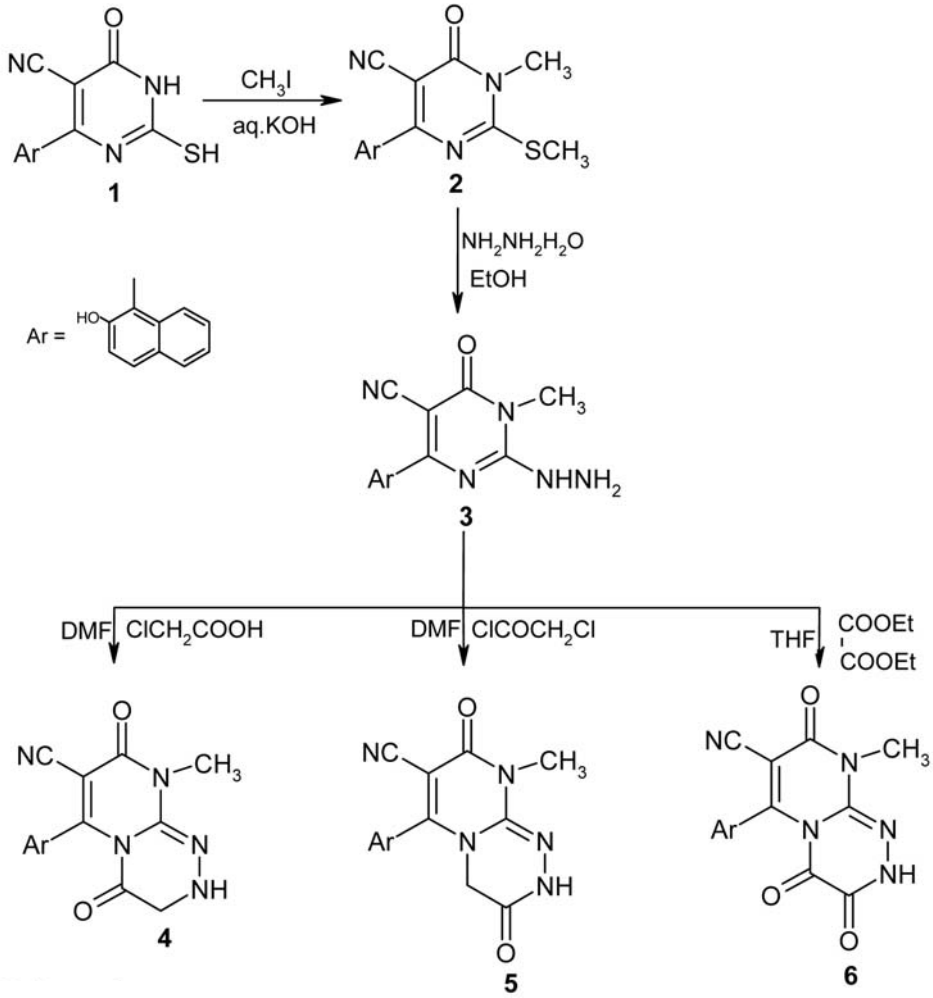
Structures of **4** and **5** were elucidated from their spectral measurements. IR spectrum of **4** showed the characteristic bands of NH, CH_2, C=O, C=N with bending of CH_2 at ν 3250, 2927, 1710, 1670, 1592 and 1468 cm^{-1}, while that of **5** showed bands attributed to NH, CH_2, C=O, C=N with bending of CH_2 at 3300, 2949, 1704, 1690, 1597 and 1472 cm^{-1} which confirm the postulated structure. ^{1}H NMR spectrum of **4** showed signals at δ 9.92, 4.28 and 3.22 ppm for the NH, CH_2 and CH_3 protons, in addition to aromatic protons at δ 7.83–7.47 ppm, while that of **5** exhibited signals at δ 9.91, 4.31 and 3.23 ppm for NH, CH_2 and CH_3 protons with aromatic protons at δ 7.78–7.39 ppm.

Heterocyclization of 2-hydrazinyl-1-methylpyrimidine **3** via refluxing with diethyl oxalate in dry THF resulted[28] in 6-(2-hydroxy-1-naphthyl)-9-methyl-3,4,8-trioxo-3,4,8,9-tetrahydro-2*H*- pyrimido[2,1-*c*][1,2,4]triazine-7-carbonitrile (**6**) (Scheme 1).

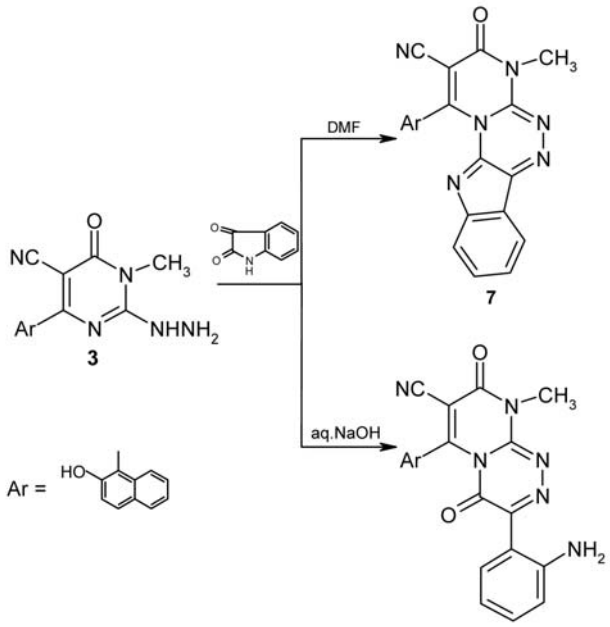
IR spectrum of **6** showed bands at 3150 cm^{-1} for NH, 1710, 1680 and 1650 cm^{-1} attributed to three C=O groups, in addition to 2920, 1480 cm^{-1} for stretching and bending of CH_3 functional group.

Behavior of isatin towards bi-nucleophilic reagents was studied to afford the heteropolycyclic systems **7** and **8**, the outcome of the reaction mainly depends on the type of the solvent used and the pH of the medium. Thus, compound **3** was refluxed with 1*H*-indole-2,3-dione in DMF affording 1-(2-hydroxy-1-naphthyl)-4-methyl-3-oxo-3,4-dihydropyrimido[2',1':3,4][1,2,4]triazino[5,6-*b*]indole-2-carbonitrile (**7**), while when the reaction was carried out in boiling aqueous sodium hydroxide produced 3-(2-aminophenyl)-6-(2-hydroxy-1-naphthyl)-9-methyl-4,8-dioxo-8,9-dihydro-4*H*-pyrimido[2,1-*c*][1,2,4]triazine-7-carbonitrile (**8**) (Scheme 2).

The structural assignment of targets **7** and **8** was deduced from spectral data. IR spectrum of **7** showed only ab-



Scheme 1



Scheme 2

sorption band at 1690 cm^{-1} for C=O with lack of NH_2 or NH groups, while that of **8** exhibited bands at 3300, 1700, 1690 cm^{-1} attributed to NH_2, two C=O functional groups. The structure of **7** was supported by its mass spectrum which showed signal at *m/z* 358 [M^+–60(–HCONHMe)], while its molecular ion peak was not observed, and the base peak at *m/z* 169 (1,2,4-triazinoindole radical, 100%) (Fig. 1).

MS spectrum of **8** recorded signal at *m/z* [M^+–137, ($C_7H_{11}N_3$)] with a base peak at *m/z* 58 (Fig. 2).

The IR spectra of all the new compounds in the solution ($CHCl_3$ used as solvent) showed the disappearance of the carbonyl functional groups between 1700–1600 cm^{-1}. This is due to a type of H-bonding and/or electrostatic attraction between the different functional groups in both the compounds and the solvent. These electronic factors confirmed the inhibition behavior of the functional groups in the new compounds obtained towards the functional groups in the tested microorganisms, resulted in the asymmetric distribution of electronic charge on the complexes-system (Fig. 3).

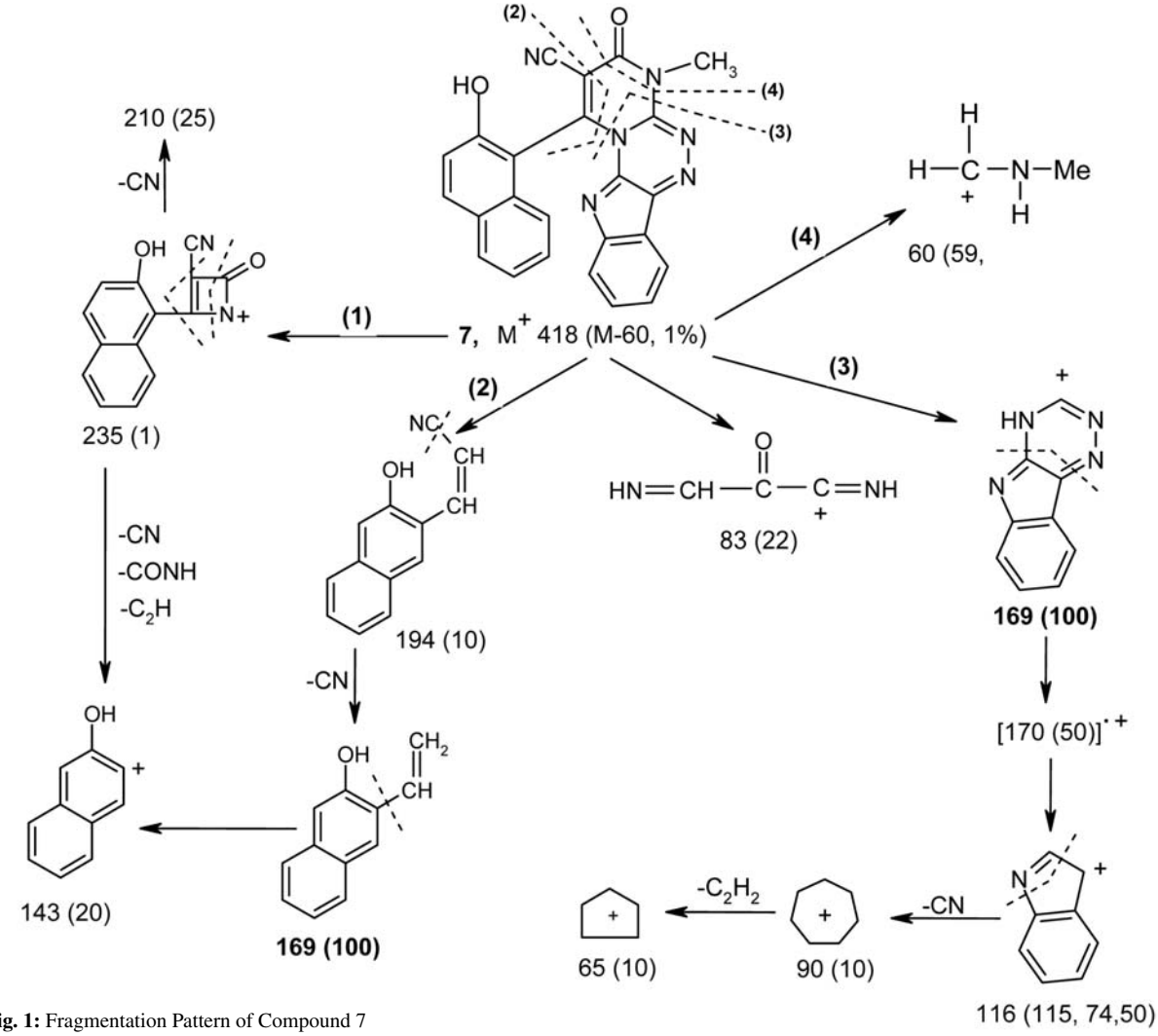


Fig. 1: Fragmentation Pattern of Compound 7

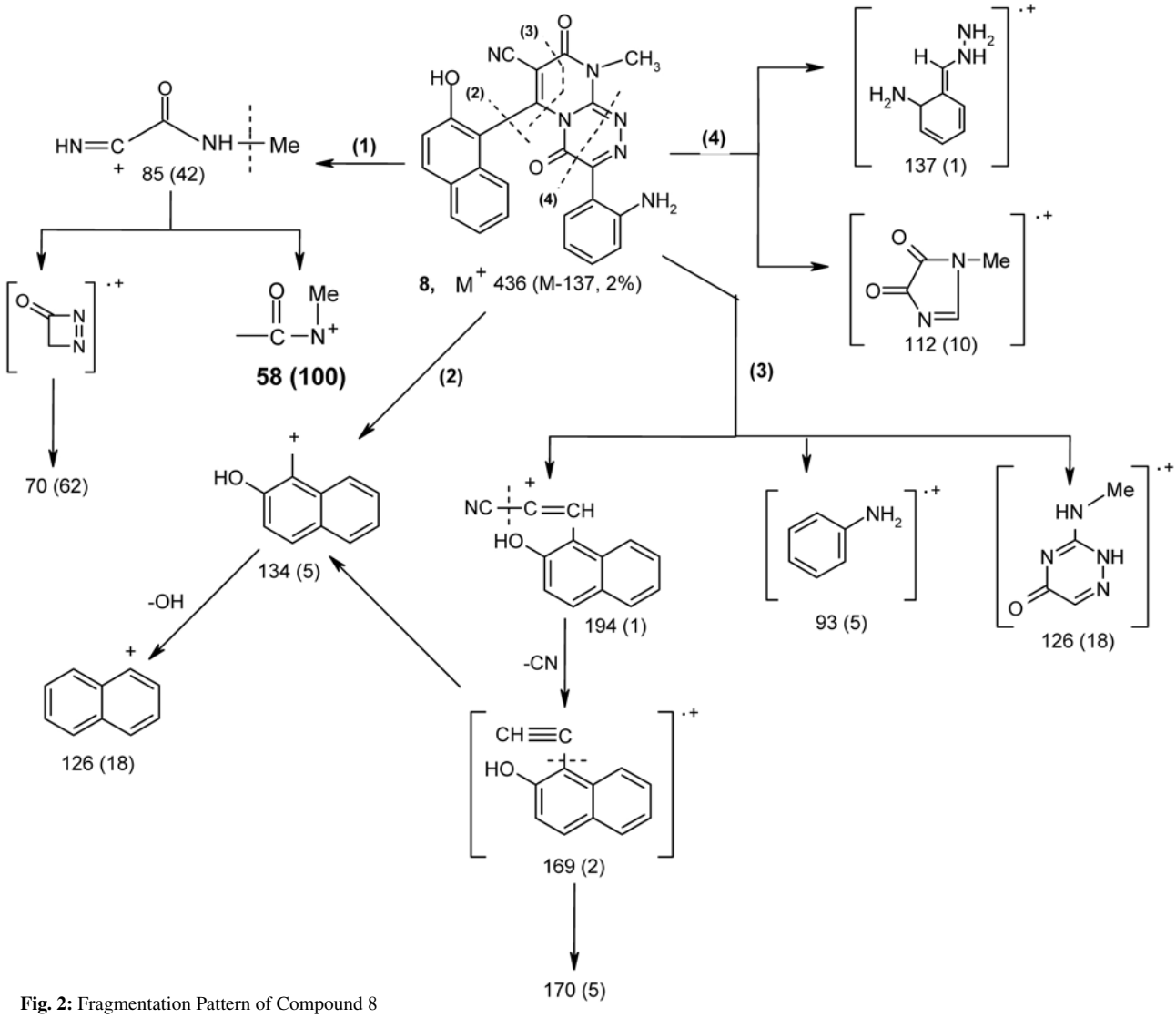


Fig. 2: Fragmentation Pattern of Compound 8

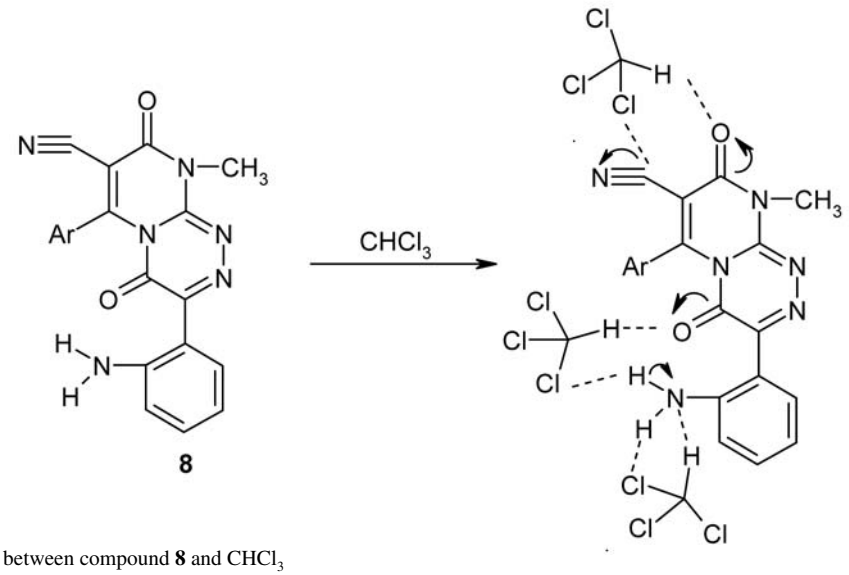
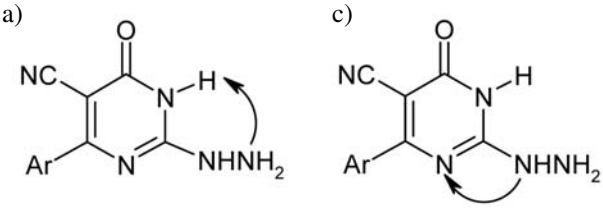


Fig. 3: The interaction between compound **8** and $CHCl_3$

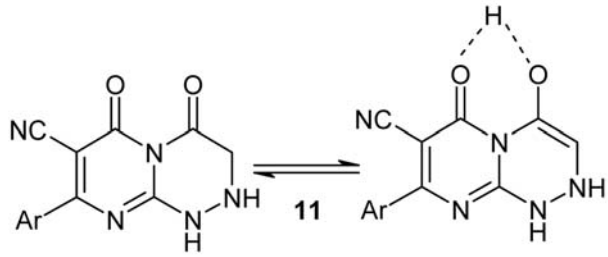
It is well know that the greater nucleophilicity of hydrazino group,[19] at the position 2 of the pyrimidine nucleus makes position 1 and/or 3 susceptible for heterocyclization via attack of various electrophilic reagents. The product of this reaction has two possible structures (**A**) and (**C**), but structure (**A**) is favorable; it can be seen that the aryl group in structure (**C**) must be twisted out of plane of the pyrimidine ring because of steric interference. This aryl group would be expected to give a compact signal in the NMR spectrum. However, the aryl group in structure (**A**) should be coplanar with the pyrimidine ring. This indicates that structure (**A**) is the correct one for the reaction product. This is in agreement with the reported NMR spectrum for other similar compounds.[29–31]

a) c)

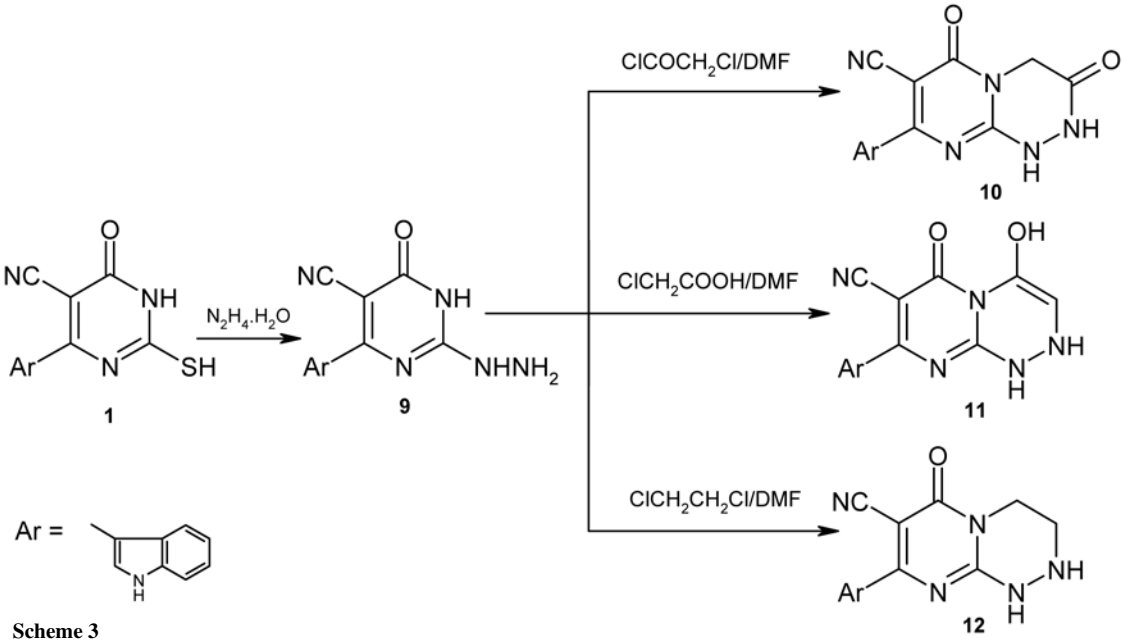


Removal of SH group from the compound **1** with hydrazine hydrate led to the formation of 4-aryl-2-hydrazino-6-oxo-1,6-dihydropyrimidin-5-carbonitrile (**9**).[27] Thus, the isomeric structures 3,6-dioxopyrimido[2,1-*c*][1,2,4] triazine **10** and 4-hydroxy-6-oxopyrimido[2,1-*c*][1,2,4] triazine **11** were isolated after treating 2-hydrazinopyrimidine **9** with chloroacetyl chloride and/or monochloroacetic acid in warm DMF. Further, reaction of 2-hydrazinopyrimidine **9** with 1,2-dichloroethane in a basic medium furnished 8-(1*H*-indol-3-yl)-6-oxo-1,3,4,6-tetrahydro-2*H*-pyrimido[2,1-*c*][1,2,4]triazine-7-carbonitrile (**12**) (Scheme 3).

Structures of both **10** and **11** can be deduced from their spectral data. UV absorption spectrum of **10** exhibited λ_{max} 405 nm, while that of **11** is 387 nm, which indicate that compound **10** contains two carbonyl groups but compound **11** contains only one carbonyl group and one hydroxyl group. IR spectrum of **10** showed two characteristic bands for C=O at 1743 and 1693 cm^{-1}, with bands at 3403 and 2911, 1449 cm^{-1} attributed to NH, stretching and bending of CH_2 groups. IR spectrum of **11** showed only characteristic band for C=O at 1673 cm^{-1}, with a band at 3602 cm^{-1} for the OH group. This confirms the stronger presence of tautomerism in the compound **11** than compound **10**. The IR pyrimidine carbonyl frequency of compound **11** is lower than that of compound **10** indicating hydrogen bonding as expected for compound **11** where the C=O and the OH groups may interact intramolecularly.



^{1}H NMR spectrum of **11** showed signals at δ 11.12 and 8.98 ppm attributed to OH and NH protons. The mass spectrometry of **10** did not exhibit a molecular ion peak but it showed a peak at *m/z* 278 attributed to [M–CO, 2%], and the base peak at *m/z* 182 ($C_{11}H_8N_3$ radical, 100%). IR spectrum of **12** displayed absorption bands at 3203, 1672 and 1496 cm^{-1} due to NH, C=O and bending of CH_2

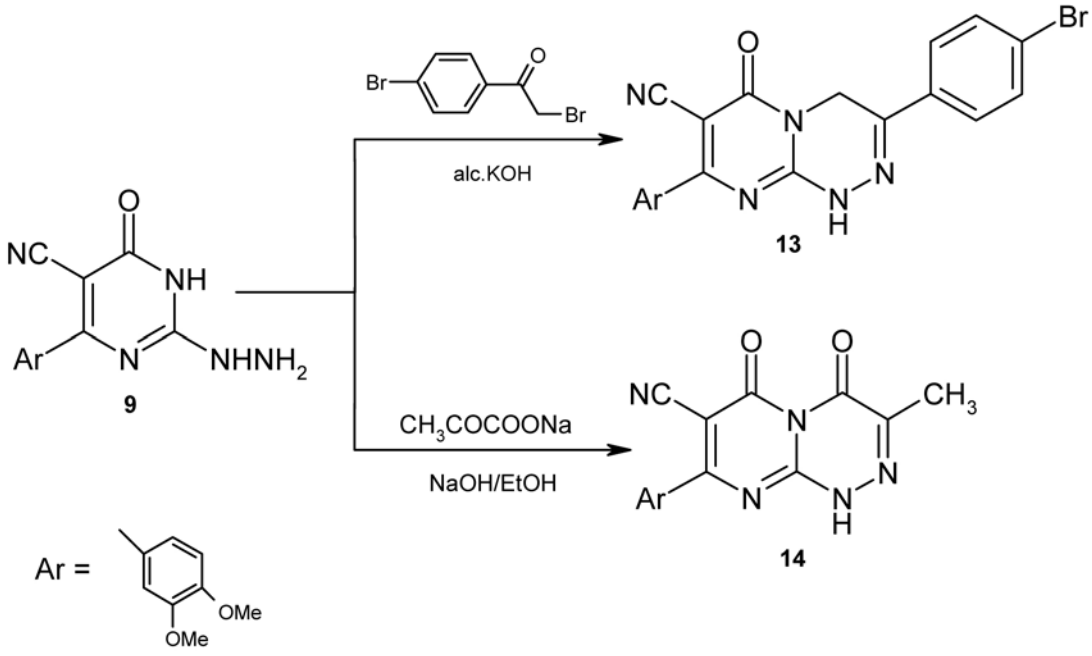


Scheme 3

groups. UV absorption spectrum of **12** showed λ_{max} at 395 nm. The mass spectrometry of **12** did not show a molecular ion peak but it exhibited a peak at *m/z* 264 attributed to [M–C_2H_4, 4%], and the base peak at *m/z* 182 ($C_{11}H_8N_3$ radical, 100%).

Perhydropyrimido[2,1-*c*][1,2,4]triazine **13** was obtained from reaction of 2-hydrazinopyrimidine **9** with active alkylating agent such as *p*-bromophenacyl bromide in boiling alkaline medium (Scheme 4). Structure of **13** was elucidated from its spectral data. UV absorption spectrum of **13** showed λ_{max} at 415 nm. IR spectrum of **13** showed the absorption bands at 3433, 1743 and 1445 cm^{-1} attributed to NH, C=O and bending of CH_2 groups. The mass spectrometry of **13** recorded a peak at *m/z* 468 (M+2, 25%) and the base peak at *m/z* 159 (*p*-bromophenyl radical, 100%).

Polyfunctional pyrimidotriazines, such as 8-(3,4-dimethoxyphenyl)-3-methyl-4,6-dioxo-1,6-dihydro-4*H*-pyrimido[2,1-*c*][1,2,4]triazine-7-carbonitrile (**14**) was also isolated from cyclocondensation of 2-hydrazinopyrimidine **9** with sodium pyruvate under reflux with sodium hydroxide solution (Scheme 4).



Scheme 4

IR spectrum of **14** showed peaks at 3289 (NH), 1732, 1662 (two C=O groups) and 1472 cm^{-1} (deformation of CH_3).

3. Biological Activities

Most of the synthesized compounds have been evaluated in vitro against Gram negative bacteria and Gram positive bacteria, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (recorded on nutrient and Maclonky agar) and also against two fungi, *Candida albicans* and *Aspergillus fumigatus* which was isolated on Sabouraud Dextrose agar (oxide) using the agar diffusion disk method.[32,33]

The antimicrobial properties of the tested compounds were established by placing presterilized filter paper disks (11 mm diameter) impregnated with 50 mg/disk using DMSO as a solvent which showed no inhibition zones (IZ) of the tested compounds. IZ were measured after 24 h incubation at 37 °C for bacteria and after 5 days incubation at 28 °C for fungi. The minimal inhibition concentrations (MIC) were estimated and recorded (Table 1). Reference antibiotics disks (11 mm in diameter) supplied by (BBL) pipericillin (PIP) and fungicide mycostatine were used for comparison.

Quantitative Structure Activity Relationship (QSAR) study for the obtained results showed that compound **7** only exhibited a higher biocidal activity by increasing of concentrations, increasing the biocidal effects (Table 2), which is due to the presence of a large number of π electrons in the conjugation with σ electrons in the fused heteropolycyclic nitrogen system. This extension of electronic conjugation decreases the electron energetic barrier from the donor to acceptor centers, which increases the electron delocalization ion through all aromatic positions leading to a higher biocidal activity. These data agree with other similar investigations.[34,35]

Table 1: The antimicrobial activities of some synthesized compounds

Tested Compounds and Standards	Inhibition Zones (at 50 mg/disk)					
	E. coli	*K. pneumoniae*	*P. aeruginosa*	*S. aureus*	*C. albicans*	*A. fumigatus*
3	14	14	14	11	14	14
7	18	18	16	22	14	14
8	14	14	14	11	14	14
P	17	17	20	27	11	11
M	11	11	11	12	34	40

P: pipericillin, M: mycostatine Highly active: IZ > 19 mm; moderately active: IZ 15–19 mm; slightly active: IZ ≈ 11–14 mm

Table 2: Minimal Inhibitory Concentration (MIC) at the highly bioactive compound 7

Compd. 7 Conc. mg/disk	Inhibition Zones (mm)			
	E. coli	*K. pneumoniae*	*P. aeruginosa*	*S. aureus*
50	18	18	16	22
40	14	12	12	20
30	11	11	11	16
20	–	–	11	12

4. Experimental Section

All melting points are uncorrected and measured using an open capillary tube by Stuart Scientific melting point SMPI (U.K). The IR spectra were recorded in the solid state as KBr discs or in $CHCl_3$ solution on a Perkin–Elmer spectrometer RXIFT-IR system No. 55529. ^{1}H and ^{13}C NMR were determined in solutions in DMSO-d_6 with a Bruker Avance DPX 400 MHz using TMS as an internal standard. Mass spectra were measured on GCMS-Q 100-EX spectrometer. Electronic absorption spectra were recorded on Shimadzu UV and visible 3101 PC spectrometer. Microanalyses were performed by the micro analytical laboratory of the Department of Chemistry, Faculty of Science, King Abdul-Aziz University, Jeddah.

Compounds **1** and **9** were prepared according to the reported methods.[27]

Synthesis of 4-(2-hydroxy-1-naphthyl)-1-methyl-2-(methylthio)-6-oxo-1,6-dihydro-pyrimidine-5-carbonitrile (2)

A mixture of compound **1** (2.95 g, 0.01 mol) and methyl iodide (1.24 mL, 0.02 mol) in aqueous KOH (1%, 100 mL) was stirred for 24 h, then filtered off, washed with EtOH and recrystallized from ethanol to give **2**. Yield 65%, mp 258–259 °C. IR (KBr) ν 3400 (OH), 3290 (NH), 2978 (CH), 2220 (CN), 1668 (CO) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 2.70 (s, 3H, SCH_3), 3.34 (s, 3H, NCH_3), 5.67 (s, 1H, OH), 7.49–7.83 (m, 6H, Ar-H). MS *m/z* 323 (M^+, 30%). Anal. Calcd for $C_{17}H_{13}N_3O_2S$: C, 63.14; H, 4.05; N, 12.99; S, 9.92. Found: C, 63.28; H, 4.00; N, 12.71, S, 9.97.

Synthesis of 2-hydrazinyl-4-(2-hydroxy-1-naphthyl)-1-methyl-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (3)

A mixture of compound **2** (3.23 g, 0.01 mol) and hydrazine hydrate (1 mL, 0.02 mol) in ethanol (100 mL) was refluxed for 4 h, cooled, then added a few drops of dil. acetic acid. The solid thus obtained was filtered off and recrystallized from EtOH–H_2O to give **3**. Yield 60%, mp 304–305 °C. IR (KBr) ν 3350 (OH), 3130 (NH), 2965 (CH), 2223 (CN), 1660 (CO) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 3.12 (s, 3H, NCH_3), 4.45 (bs, 2H, NH_2), 5.57 (s, 1H, OH), 7.48–7.79 (m, 6H, Ar-H), 8.93 (s, 1H, NH). MS *m/z* 307 (M^+, 25%). Anal. Calcd for $C_{16}H_{13}N_5O_2$: C, 62.53; H, 4.26; N, 22.79. Found: C, 63.00; H, 4.28; N, 22.23.

Synthesis of 6-(2-hydroxy-1-naphthyl)-9-methyl-4,8-dioxo-3,4,8,9-tetrahydro-2*H*-pyrimido[2,1-*c*][1,2,4]triazine-7-carbonitrile (4)

A mixture of compound **3** (3.07 g, 0.01 mol) and monochloroacetic acid (0.945 g, 0.01 mol) in DMF (50 mL) was refluxed for 2 h, cooled, then poured onto ice. The obtained solid was filtered off and recrystallized from DMF–H_2O to give **4**. Yield 70%, mp 278–280 °C. IR (KBr) ν 3440 (OH), 3250 (NH), 2927 (CH), 2222 (CN), 1710, 1670 (CO) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 3.22 (s, 3H, NCH_3), 4.28 (s, 2H, CH_2), 5.29 (s, 1H, OH), 7.47–7.83 (m, 6H, Ar-H), 9.92 (s, 1H, NH). Anal. Calcd for $C_{18}H_{13}N_5O_3$: C, 62.24; H, 3.77; N, 20.16. Found: C, 62.20; H, 3.74; N, 20.11.

Synthesis of 6-(2-hydroxy-1-naphthyl)-9-methyl-3,8-dioxo-3,4,8,9-tetrahydro-2*H*-pyrimido[2,1-*c*][1,2,4] triazine-7-carbonitrile (5)

To a solution of compound **3** (3.07 g, 0.01 mol) in DMF (50 mL), chloroacetyl chloride (0.79 mL, 0.01 mol) was added drop wise. The solid thus obtained was filtered off and recrystallized from DMF–H_2O to give **5**. Yield 65%, mp 298–300 °C. IR (KBr) ν 3410 (OH), 3300 (NH), 2949 (CH), 2225 (CN), 1704, 1690 (CO) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 3.23 (s, 3H, NCH_3), 4.31 (s, 2H, CH_2), 5.28 (s, 1H, OH), 7.39–7.78 (m, 6H, Ar-H), 9.91 (s, 1H, NH). MS *m/z* 347 (M^+, 5%). Anal. Calcd for $C_{18}H_{13}N_5O_3$: C, 62.24; H, 3.77; N, 20.16. Found: C, 62.28; H, 3.76; N, 19.98.

Synthesis of 6-(2-hydroxy-1-naphthyl)-9-methyl-3,4,8-trioxo-3,4,8,9-tetrahydro-2*H*-pyrimido[2,1-*c*][1,2,4]triazine-7-carbonitrile (6)

Equimolar amounts of compound **3** (3.07 g, 0.01 mol) and diethyl oxalate (1.35 mL, 0.01 mol) in THF (50 mL) were refluxed for 12 h and then cooled. The solid so obtained was filtered off and recrystallized from dioxane to give **6**. Yield 65%, mp 316–317 °C. IR (KBr) ν 3450 (OH), 3150 (NH), 2920 (CH), 2216 (CN), 1710, 1680, 1650 (CO) cm^{-1}. ^1H NMR (DMSO-d_6) δ 3.18 (s, 3H, NCH_3), 5.19 (s, 1H, OH), 7.38–7.85 (m, 6H, Ar-H), 9.53 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 167.1, 163.1, 162.5, 161.4, 155.3, 153.7, 131.8, 130.0, 128.4, 126.5, 123.4, 122.1, 119.7, 116.7, 115.5, 76.5, 24.8. MS *m/z* 361 (M^+, 12%). Anal. Calcd for $C_{18}H_{11}N_5O_4$: C, 59.84; H, 3.07; N, 19.38. Found: C, 59.78; H, 3.04; N, 19.15.

Synthesis of 1-(2-hydroxy-1-naphthyl)-4-methyl-3-oxo-3,4-dihydropyrimido[2',1':3,4][1,2,4]triazino[5,6-*b*]indole-2-carbonitrile (7)

A mixture of compound **3** (3.07 g, 0.01 mol) and isatin (1.47 g, 0.01 mol) in DMF (50 mL) was refluxed for 4 h, cooled, then poured onto crushed ice. The produced solid was filtered off and recrystallized from DMF to give **7**. Yield 71%, mp 306–308 °C. IR (KBr) ν 3420 (OH), 2950 (CH), 2230 (CN), 1690 (CO) cm^{-1}. ^1H NMR (DMSO-d_6) δ 3.23 (s, 3H, NCH_3), 5.54 (s, 1H, OH), 7.18–7.99 (m, 10H, Ar-H). Anal. Calcd for $C_{24}H_{14}N_6O_2$: C, 68.89; H, 3.37; N, 20.09. Found: C, 68.88; H, 3.34; N, 19.66.

Synthesis of 3-(2-aminophenyl)-6-(2-hydroxy-1-naphthyl)-9-methyl-4,8-dioxo-8,9-dihydro-4*H*-pyrimido[2,1-*c*][1,2,4]triazine-7-carbonitrile (8)

To a solution of isatin (1.47 g, 0.01 mol) in aqueous NaOH (5%, 100 mL), warmed for 5 min, compound **3** (3.07 g, 0.01 mol) was added and refluxed for 2 h, cooled, then poured onto ice with HCl (36%, 5 mL) for neutralization. The resulting solid was filtered off and recrystallized from EtOH to give **8**. Yield 80%, mp 310–312 °C. IR (KBr) ν 3450 (OH), 3300, 3230 (NH_2), 2930 (CH), 2205 (CN), 1700, 1690 (CO) cm^{-1}. ^1H NMR (DMSO-d_6) δ 3.18 (s, 3H, NCH_3), 4.27 (bs, 2H, NH_2), 5.36 (s, 1H, OH), 7.29–7.87 (m, 10H, Ar-H). Anal. Calcd for $C_{24}H_{16}N_6O_3$: C, 66.05; H, 3.70; N, 19.26. Found: C, 66.20; H, 3.68; N, 19.06.

Synthesis of 8-(1*H*-indol-3-yl)-3,6-dioxo-1,3,4,6-tetrahydro-2*H*-pyrimido[2,1-*c*][1,2,4]triazine-7-carbonitrile (10)

To a solution of compound **9** (2.66 g, 0.01 mol) in DMF (30 mL), chloroacetyl chloride (0.79 mL, 0.01 mol) was added drop wise at room temperature. The mixture was refluxed for 2 h, cooled, then poured onto ice. The precipitate was filtered off and recrystallized from THF to give **10**. Yield 85%, mp 240–241 °C. IR (KBr) ν 3403 (NH), 2911 (CH), 2218 (CN), 1743, 1693 (CO) cm^{-1}. ^1H NMR (DMSO-d_6) δ 4.42 (s, 2H, CH_2), 7.38–8.57 (m, 5H, Ar-H), 9.24 (s, 2H, 2NH), 10.90 (bs, 1H, NH of indole). Anal. Calcd for $C_{15}H_{10}N_6O_2$: C, 58.82; H, 3.29; N, 27.44. Found: C, 58.80; H, 3.30; N, 27.17.

Synthesis of 4-hydroxy-8-(1*H*-indol-3-yl)-6-oxo-1,6-dihydro-2*H*-pyrimido[2,1-*c*][1,2,4]triazine-7-carbonitrile (11)

Equimolar amounts of compound **9** (2.66 g, 0.01 mol) and monochloroacetic acid (0.945 g, 0.01 mol) in DMF (30 mL) were refluxed for 4 h, then poured onto ice. The separated solid was filtered off and recrystallized from dioxane to give **11**. Yield 73%, mp 268–270 °C. IR (KBr) ν 3602 (OH), 2215 (CN), 1673 (CO) cm^{-1}. ^1H NMR (DMSO-d_6) δ 7.29–8.38 (m, 6H, Ar-H), 8.98 (s, 2H, 2NH), 10.36 (bs, 1H, NH of indole), 11.12 (s, 1H, OH). Anal. Calcd for $C_{15}H_{10}N_6O_2$: C, 58.82; H, 3.29; N, 27.44. Found: C, 58.78; H, 3.28; N, 26.90.

Synthesis of 8-(1*H*-indol-3-yl)-6-oxo-1,3,4,6-tetrahydro-2*H*-pyrimido[2,1-*c*][1,2,4]triazine-7-carbonitrile (12)

A mixture of compound **9** (2.66 g, 0.01 mol) and 1,2-dichloroethane (0.78 mL, 0.01 mol) in DMF (30 mL) was refluxed for 2 h, then poured onto crushed ice. The solid which separated was filtered off and dried, then recrystallized from THF to give **12**. Yield 65%, mp 300–301 °C. IR (KBr) ν 3203 (NH), 2920 (CH), 2220 (CN), 1672 (CO) cm^{-1}. ^1H NMR (DMSO-d_6) δ 4.58 (s, 4H, CH_2CH_2), 7.29–8.48 (m, 5H, Ar-H), 9.56 (s, 2H, 2NH), 10.70 (bs, 1H, NH of indole). Anal. Calcd for $C_{15}H_{12}N_6O$: C, 61.64; H, 4.14; N, 28.75. Found: C, 61.58; H, 4.18; N, 28.32.

Synthesis of 3-(4-bromophenyl)-8-(3,4-dimethoxyphenyl)-6-oxo-1,6-dihydro-4*H*-pyrimido[2,1-*c*][1,2,4]triazine-7-carbonitrile (13)

A mixture of compound **9** (2.66 g, 0.01 mol) and *p*-bromophenacyl bromide (2.77 g, 0.01 mol) in ethanolic KOH (5%, 50 mL) was heated under reflux for 4 h, cooled, then added dil. HCl. The solid thus formed was filtered off and washed with K_2CO_3 (1%, 100 mL) then recrystallized from EtOH to give **13.** Yield 80%, mp 200–202 °C. IR (KBr) ν 3433 (NH), 2211 (CN), 1743 (CO) cm^{-1}. ^1H NMR (DMSO-d_6) δ 3.82 (s, 6H, OCH_3), 4.15 (s, 2H, CH_2), 6.98–8.72 (m, 7H, Ar-H), 9.16 (s, 1H, NH). Anal. Calcd for $C_{21}H_{16}BrN_5O_3$: C, 54.09; H, 3.46; N, 15.02. Found: C, 53.88; H, 3.40; N, 14.86.

Synthesis of 8-(3,4-dimethoxyphenyl)-3-methyl-4,6-dioxo-1,6-dihydro-4*H*-pyrimido[2,1-*c*][1,2,4]triazine-7-carbonitrile (14)

Equimolar amounts of compound **9** (2.66 g, 0.01 mol) and sodium pyruvate (1.10 g, 0.01 mol) in ethanolic NaOH (5%, 50 mL) were refluxed for 2 h, cooled, then

poured onto ice with HCl (36%, 10 mL). The resulting solid was filtered off, washed with cold water, dried and recrystallized from AcOH to give **14**. Yield 75%, mp 168–170 °C. IR (KBr) ν 3289 (NH), 2209 (CN), 1732, 1662 (CO) cm^{-1}. ^1H NMR (DMSO-d_6) δ 2.87 (s, 3H, CH_3), 3.98 (s, 6H, OCH_3), 7.49–8.76 (m, 3H, Ar-H), 8.84 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 169.5, 160.0, 165.5, 162.3, 154.8, 149.4, 130.1, 119.6, 115.3, 111.5, 92.9, 55.7, 15.3. MS *m/z* 339 (M^+, 3%). Anal. Calcd for $C_{16}H_{13}N_5O_4$: C, 56.64; H, 3.86; N, 20.64. Found: C, 56.70; H, 3.84; N, 20.22.

5. Conclusions

2-Hydrazinyl-1-methylpyrimidine **3** and 2-hydrazinopyrimidine **9** were found to be good intermediates for the synthesis of various pyrimido-1,2,4-triazine derivatives. Some of them were tested for their biocidal effects.

6. References

1. A. T. Balaban, D. C. Oniciu, A. R. Katrizky, *Chem. Rev*. **2004**, *104*, 2777–2812.
2. C. Mugnaini, M. Alorgi, A. Togninelli, H. Gevariya, A. Brizzi, F. Manetti, C. Bernardini, L. Angeli, A. Tafi, L. Bellucci, F. Corelli, S. Massa, G. Maga, A. Samuele, M. Facchini, I. Clotet-Codina, M. Armand-Ugón, J. A. Esté, M. Botta, *J. Med. Chem*. **2007**, *50*, 6580–2595.
3. W. Mo, G. Liao, T. Wang, H. He, *J. Fluorine Chem*. **2008**, *129*, 519–523.
4. J. L. Misset, *Rev. Prat.* 1997, *47*, 29–35.
5. M. D. Varney, C. L. Palmer, W. H. Romines III, T. Boritzki, S. A. Margosiak, R. Almassy, C. A. Janson, C. Bartlett, E. J. Howland, R. Ferre, *J. Med. Chem.* **1997**, *40*, 2502–2524.
6. E. Bajetta, C. Carnaghi, L. Somma, C. G. Stampino, *Tumori* **1996**, *82*, 450–452.
7. F. Bondavalli, O. Bruno, A. Ranise, P. Schenone, F. Rossi, *Farmaco* **1992**, *47*, 171–190.
8. O. Bruno, A. Ranise, F. Bondavalli, P. Schenone, F. Rossi, *Farmaco* **1993**, *48*, 949–966.
9. J. Feeney, *Arch. Dermatol*. **1997**, *133*, 1173.
10. R. Garg, S. P. Gupta, *J. Enzyme-Inhib*. **1997**, *11*, 171–181.
11. P. Sargent, D. J. Williamson, G. Pearson, J. Odontiadis, P. J. Cowen, *Psychopharmacology* **1997**, *132*, 296–302.
12. K. A. El-Bayouki, W. M. Basyouni, F. A. Gad, M. M. El-Sayed, *Egypt. J. Pharm. Sci*. **1992**, *33*, 517–526.
13. W. M. Basyouni, Z. E. El-Bazza, *Egypt. J. Pharm. Sci*. **1992**, *33*, 503–515.
14. F. A. Ashour, N. S. Habib, M. El-Taibbi, S. El-Dine, A. S. El-Dine, *Farmaco* **1990**, *45*, 1341–1349.
15. A. Gupta, L. Prakash, *Boll. Chim. Farm.* **1994**, *133*, 163–166.
16. E. B. Moawad, M. Y. Yousif, M. A. Metwally, *Pharmazie* **1989**, *44*, 820–822.
17. C. O. Kappe, T. Kappe, *Arch. Pharm. Weinheim-Ger*. **1991**, *324*, 863–866.
18. R. M. Abdel-Rahman, *Trends Heterocycl. Chem*. **2002**, *8*, 187–195.
19. R. M. Abdel-Rahman, W. R. Abdel-Monem, *Indian J. Chem*. **2007**, *46B*, 838–846.
20. M. A. Ibrahim, R. M. Abdel-Rahman, A. M. Abdel-Halim, S. S. Ibrahim, H. A. Allimony, *J. Braz. Soc.* **2009**, *20*, 1275–1286.
21. A. T. Ramadan, R. M. Abdel-Rahman, M. A. El-Behairy, A. I. Ismail, M. M. Mahmoud, *Thermochimica Acta* **1993**, *222*, 291–303.
22. (a) R. M. Abdel-Rahman, *Pharmazie* **2001**, *56*, 18–30. (b) R. M. Abdel-Rahman, *Pharmazie* **2001**, *56*, 195–212. (c) R. M. Abdel-Rahman, *Pharmazie* **2001**, *56*, 275–286.
23. W. R. Abdel-Monem, R. M. Abdel-Rahman, *International J. Chem.* **2006**, *16*, 1–6.
24. T. E. Ali, R. M. Abdel-Rahman, F. I. Hanafy, S. M. El-Edfawy, *Phosphorus, Sulfur and Silicon* **2008**, *183*, 2565–2577.
25. M. A. Ibrahim, R. M. Abdel-Rahman, A. M. Abdel-Halim, H. A. Allimony, *ARKIVOC* **2008**, (*xvi*), 202–215.
26. M. Abdel-Megid, M. Abdel-Hamid, K. M. El-Mahdy, M. Seada, M. M. Elsayed, *J. Chem. Chem. Eng.* **2010**, *4*, 41–53.
27. S. A. Abdel-Aziz, H. A. Allimony, H. M. El-Shaaer, U. F. Ali, R. M. Abdel-Rahman, *Phosphorus, Sulfur and Silicon* **1996**, *113*, 67–77.
28. R. M. Abdel-Rahman, J. M. Morsy, H. A. Allimony, W. R. Abdel-Monem, *Boll. Chim. Farm.* **1999**, *138*, 176–178.
29. N. S. Bhacca, L. Johnson, J. Shoolery, *Nuclear Magnetic Resonance Spectra Catalog*, Spectra Nos. 305, 306, Varian Associates, Palo Alto **1962**.
30. L. A. Lee, J. W. Wheeler, *J. Org. Chem*. **1972**, *37*, 348–351.
31. H. A. Daboun, A. M. El-Reedy, *Z. Naturforsch*. **1983**, *38b*, 1686–1689.
32. M. J. Weinstein, G. H. Nagman, »Antibiotics« Elsevier Scientific Publishing Company, Amsterdam**, 1978**, 464.
33. A. L. Barry, C. Thornberry, Susceptibility testing diffusion test procedures. In manual of clinical microbiology, E.H. Lennetl, W. J. Hausler, J. P. Truant, 6th Ed. Washington D. C., American Society for Microbiology, **1981**, 561.
34. C.-C. Zhao, Q.-X. Tong, Z.-T. Li, L.-Z. Wu, L.-P. Zhang, C.-H. Tung, *Tetrahedron Lett.* **2004**, *45*, 6807–6811.
35. T. E.-S. Ali, *Eur. J. Med. Chem*. **2009**, *44*, 4385–4392.

Povzetek

S pomočjo ciklizacijskih reakcij 1-metil-2-hidrazinopirimidina **3** in/ali 2-hidrazinopirimidina **9** z acikličnimi oz. cikličnimi kisikovimi spojinami smo pod različnimi pogoji pripravili nekatere nove izomerne strukture pirimido[2,1-*c*][1,2,4]triazinov **4**–**8** in **10**–**14**. Strukture pripravljenih spojin smo potrdili z elementno analizo in s pomočjo spektroskopskih podatkov (UV, IR, $^{1}H/^{13}C$ NMR in masna spektrometrija). Večini pripravljenih spojin smo tudi določili morebitno antimikrobno aktivnost in jo primerjali s standardnima antibiotikoma pipericilinom in mikostatinom. Le spojina **7** je kazala biocidni učinek.