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Synthesis of Some New Fluorinated Fused Heteropolycyclic Nitrogen Systems Containing pyrazolo[3,4-d]pyrimidines Moiety and Their Effects on Cellobiase Activity Produced by *Aspergillus nidulans* Fungi

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Abstract. New Fluorinated fused heteropolycyclic nitrogen systems containing pyrazolo[3,4-d] pyrimidines moieties have been synthesized from the ring closure reaction of the corresponding hydrazine derivatives with bifunctional compounds. Structure of the new fluorinated systems obtained has been established from elemental and spectral analysis. The effect of these compounds was evaluated on the activity of cellobiase produced by *Aspergillus nidulans* fungi.

Keywords: Pyrimidines, fluoropyrazol, cellobiase activity, hydrazine derivatives.

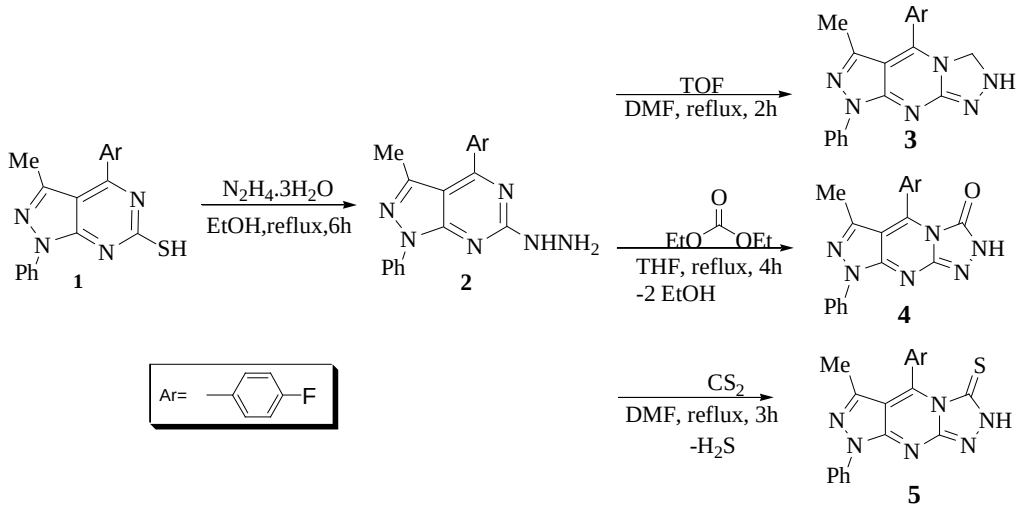
1 Introduction

Recently, Hydrazine group bearing 1,2,4-triazines are used to synthesize an important heterocyclic nitrogen system with various functional group [1], in addition both the pyrazoles [2] and pyrimidines [3] exhibit medicinal, pharmacological and biological activities. The introduction of fluorine C-F and CF_3 group to heterocyclic systems often improves and enhances their properties. Thus, this work tends to synthesize some more new fluorinated fused heteropolycyclic nitrogen systems containing pyrazolo[3,4-d]pyrimidines moieties starting from hydrazine-pyrazolopyrimidines, in view of their effects on the cellobiase activity produced by *Aspergillus nidulans* fungi.

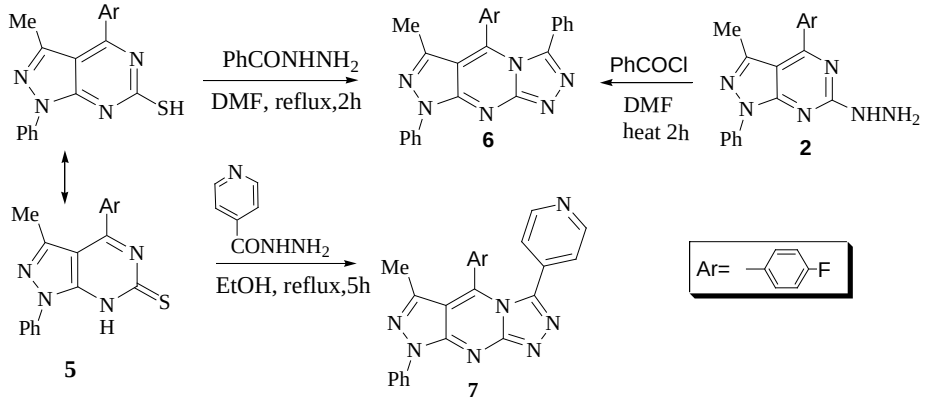
2. Chemistry

The 6-hydrazino-4-(4‘-fluorophenyl)-3-methyl-1-phenylpyrazolo[3,4-d]pyrimidines (2) were obtained from hydrazinolysis of compound 1 [4]. Pyrimidine 2 is used as a starting material for building a number of fused polyheterocyclic nitrogen systems. Thus, ring closure reactions of compound 2 with triethyl orthoformat (TOF) in reflux tetrahydrofuran (THF) produced the pyrazolo[4‘,3‘:5,6]pyrimido[2,3-d][1,2,4]triazole 3, while the reaction with diethylcarbonate or carbon disulfide in reflux DMF furnished pyrazolo[4‘,3‘:5,6]pyrimido[2,3-c][1,2,4]triazole-6-one 4 and pyrazolo[4‘,3‘:5,6]pyrimido[2,3-c][1,2,4]triazole -6-thione 5 (Scheme 1).

In addition, fused heteropolycyclic nitrogen systems 6 and 7 were obtained from the reaction of 1 with benzoic acid hydrazide or isonicotinic acid hydrazine in DMF under reflux (scheme 2). The nucleophilic attack of NH_2 of acid hydrazide was followed by ring closure reaction by elimination of H_2O molecule (figure 1).



Scheme 1. Synthesis of triazole-6-one **4** and triazole-6-thione **5**.



Scheme 2. Synthesis of fused heteropolycyclic nitrogen systems 6 and 7.

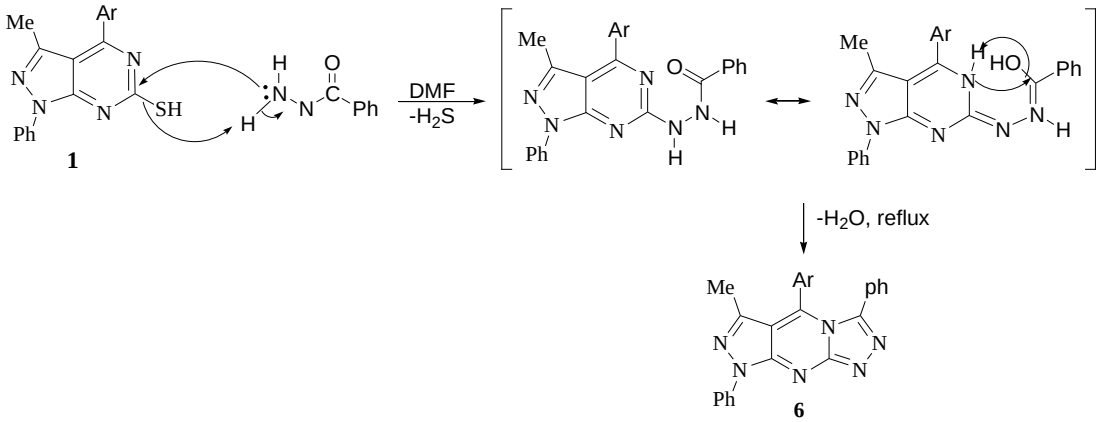
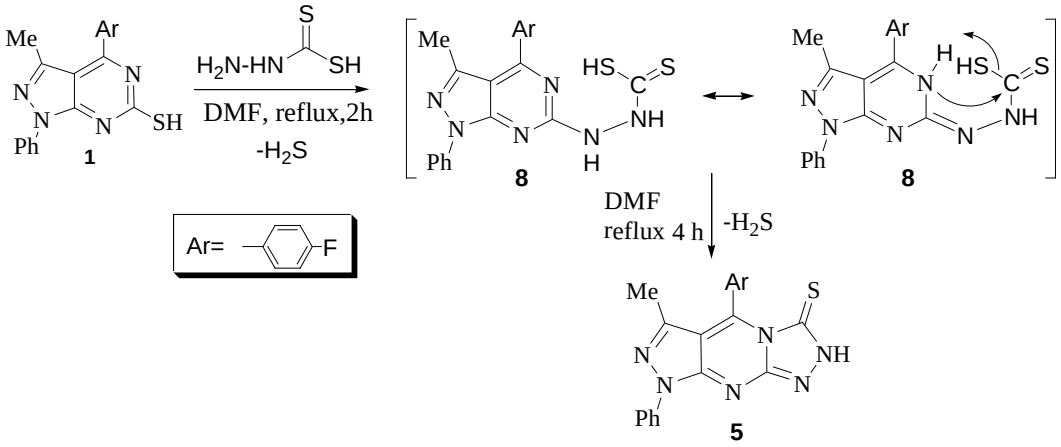


Figure 1. Suggested formation of compound 6.

The nucleophilic attack of -NH_2 dithioic formic acid hydrazide on mercapto group of compound 1 in DMF under reflux for 2 h gave *N*-substituted-thiohydrazide 8. [5] The further Heterocyclization of compound 8 furnished compound 5 (scheme 3).

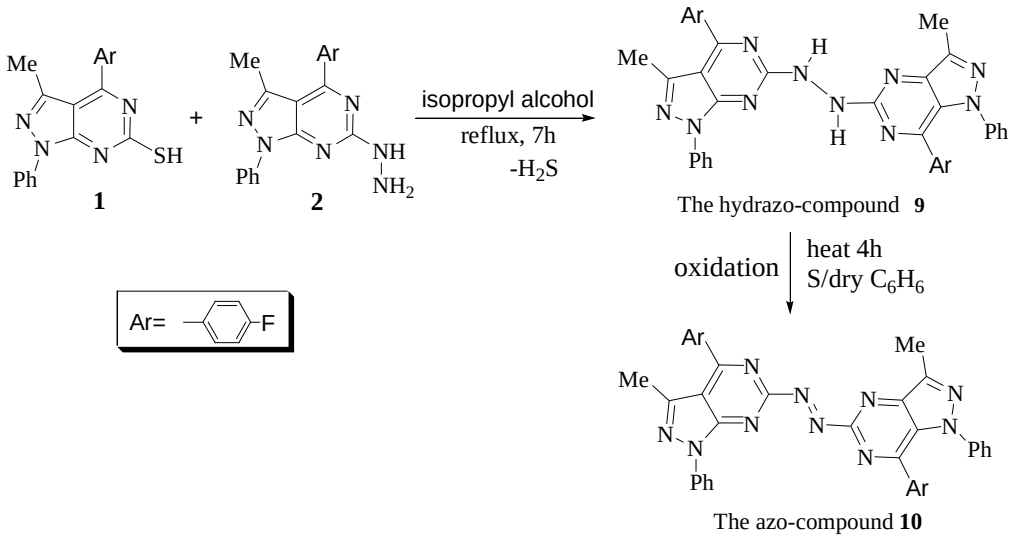


Scheme 3. Synthesis of N-substituted-thiohydrazide 8.

The hydrazo and azo compounds exhibit an important attention due to its application in the industry and agriculture field [6,7]. Thus, interaction between compounds 1 and 2 in isopropyl alcohol under reflux afforded the hydrazo-derivative 9, which upon simple oxidation gave the azo compound 10. (Scheme 4).

3. Result and Discussion

The Ultraviolet (UV) spectrum for synthesis compounds 2, 3 and 4 showed λmax at 430 (1.79), 437 (0.35) and 540 (0.85) nm. The fused heteropolycyclic nitrogen system bears both a chromophor and oxo-chrom probes. In addition, the hetero-conjugation systems formed enhance the λ_{max} of compound 4 in comparison with compound 2. The IR spectroscopy spectrum of new synthesized systems showed lakes of $-NH_2$ functional group of 1,2,4-triazin at 3300 cm^{-1}, compound 4 showed an interesting type of enol-keto form systems exhibited at ν1650-1620 cm-1. On the other hand, compounds 5 and 8 recorded two functional groups NH and C=S at ν 3300-3100 and 1180 respectively.



Scheme 4. Synthesis of azo compound 10 via hydrazo-derivative 9 oxidation.

The [1]HNMRspectrum of both compounds 4, 5 and 10 showed two broad singlet for NH proton at δ 9 and 3.5 ppm, while compound 8 exhibited two different singlet for NH and SH protons at δ 8.5 and 4.8 ppm respectively. Compounds 3, 6, 7 and 10 showed a lack of NH peaks.

Additionally, [13]CNMR spectrum showed as expected a various aliphatic and aromatic carbons at 14, 129-125 and C-F, C=N, C-N and C-C carbons at δ 144, 141, 110 ppm respectively. Only compounds 4 and 5 showed carbonyl C=O and thionyl carbon C=S carbons at δ 152 and 162 ppm.

The mass fragmentation pattern of the fluorinated heterocyclic systems exhibited a base peak at *m/e* 95(100%), attribute to 4-fluorophenyl radicals.

4. Experiments

Melting points determined with an electrochermal Bibly Sturat Scientific melting Point sample (UK). A Perkin Elmer Model RXI-FT IR system 55529 was used for Recording IR spectra of the prepared compounds (cm-1). A Bruker advance DPX 400 MHZ model using TMS as internal standard was used for recording the [1]H and [13]CNMR spectra of the compounds on DMSO-d_6 (ppm). A GC-MS-GP 1000 Ex model was used for recording the mass spectra of the compounds (MHz). Electronic spectra recorded in ethanol on Shimadzu UV and visible 310 IPC Spectrophotometer (nm). Elemental analysis was performed in micro analytical Center of Cairo University, Cairo, Egypt.

6-Hydrazino-4-(4`-fluorophenyl)-1-phenyl-3-methyl-pyrazolo[3,4d]pyrimidines (2)

A mixture of 5 (2g, 6.0 mmol) and hydrazine hydrate (100%, 0.516g, 6.0 mmol) in EtOH (40 ml) refluxed for 8 h, cooled. The resulting solid collected by filtration and crystallized from EtOH to give compound 2 as orang crystals. Yield 93%, m.p. 228-230 ºC. Analytical data; Found: C,64.51; H, 4.30; N,24.88 % . Calculated for $C_{18}H_{15}N_6F$ (334); C,64.67; H, 4.49; N, 25.01 %. UV (nm) λ_{max}: 430 (ε 1.79). IR (cm-1) ν: 3300, 3100 (NH_2,NH), 2919 (aliph. CH), 1620 (deformation NH_2), 1610 (C=C), 1591 (C=N), 1497 (deformation of CH_3), 1217(C-F), 903, 787(ph). [1]HNMR (ppm) δ: 9.53 (s,1H,NH), 7.92-7.82 (m,4H, ar), 7.78-7.55 (s,1H,ar), 7.39-7.25 (d, 2H,ar), 1,24 (s,3H, CH3). [13]CNMR (ppm) δ: 144 (C-F), 129.19 (C=N), 129.07, 128.94, 128.89, 127.68, 126.66, 126.03(ar carbons, 99.66 (C-N), 14.14(C-CH3). *m/s* = 352(M+ H2O, 1%), 95(100%).

1-Phenyl-3-methyl-4-(4`-fluorophenyl)pyrazolo[4`,3`:5,6]pyrimido[2,3-c][1,2,4]triazole (3)

A mixture of 14 (0.4g, 1.0 mmol) and TOF (0.148g, 1.0 mmol) in DMF (27 ml) refluxed for 2h and cooled. The resulting solid collected by filtration and crystallized from DMF to give compound 3. Yield 92%, m.p.192-194 ºC. Analytical data; Found: C,66.00; H, 3.51; N,24.12 %. Calculated for $C_{19}H_{13}N_6F$ (334); C,66.27; H, 3.77; N, 24.41%. UV (nm) λ_{max}: 430 (ε 1.79). IR (cm-1) ν: 2920, 2850 (aliph.CH), 1592, 1582 (C=N), 1492(deformation of CH_3), 1220 (C-F), 903,788 (ph).

4-Aryl-3-methyl-1-phenyl-1-pyrazolo[4`,3`:5,6]pyrimido[2,3-c][1,2,4]triazole-6-(7H)one (4)

A mixture of 14 (0.6g, 0.02 mol) and diethyl carbonate (0.236g, 0.002 mol) with tetrahydrofuran THF (30 ml) refluxed for 4h, cooled. The resulting solid collected by filtration and crystallized from THF to give compound 4 as pale yellow crystal. Yield 91%, m.p. 220-221 ºC. Analytical data; Found: C,62. 98;H, 3.55; N,23.01%. Calculated for $C_{19}H_{14}N_6FO$ (361); C,63.15; H, 3.87; N, 23.26 %. IR (cm-1) γ: 3090 (NH), 2919, 2849 (aliph. CH), 1650 (C=O) 1620, 1592,1582 (C=N), 1458 (deformation CH3), 1219 (C-F), 903,831 (ph). IR (cm-1) ν: 3200-3400 (b, NH), 2920 (aliph. CH), 1592,1582 (C=N), 1498 (deformation CH3), 1366 (NCN), 1221 (C-F), 902,831 (ph). 1HNMR (ppm) δ: 7.90-7.915, 7.84-7.85 (each d, 2H, ar), 7.45-7.46 (m,5H, ar), 7.30, 7.28-7.29, 7.25-7.27(each s,2H, ar), 4.4 (s,1H,OH), 1,25 (s, 3H, CH_3).

4-Aryl-3-methyl-1-phenyl-1-pyrazolo[4`,3`:5,6]pyrimido[2,3-c][1,2,4]triazole-6-(7H)thion (5)

A mixture of 14 (0.2g, 0. 6 mmol) and CS_2 (0.14g, 0. 6 mmol) in DMF (20 ml) refluxed for 3h, cooled. The resulting solid collected by filtration and crystallized from DMF to give compound 5 as yellow crystals. Yield 88%, m.p. 184-185 ºC. Analytical data; Found: C,60.31; H, 3.55; N,22.00; S, 8.21 %. Calculated for $C_{19}H_{14}N_6FS$ (377); C,60.47; H, 3.71; N, 22.28; S, 8.48%. IR (cm-1) γ: 3080 (NH), 2850,2920 (aliph. CH), 1592,1608 (C=N), 1450,1498 (deformation of CH_3), 1365 (NCSN), 1260 (C-F), 1168(C=S), 788,903 (ph). [1]HNMR (ppm) δ: 9.53 (s,1H,NH), 7.90-7.98, 7.86-7.87 (each d,2H, ar), 7.81-7.85, 7.60-7.79 (each s,2H,aromatic), 7.40-7.48 (m,4H,ar), 7.31-7.37, 7.29-7.30 (each d,2H, ar), 1.24 (s,3H,CH_3). [13]CNMR (ppm) δ: 161.28 (C=S),138 (C-F),137 (C-F), 129.19 (C=N), 121.17, 126.64, 128.93 (ar-C), 109 (C-C),12.93 (C-CH3). m/s (Int.y.): 379 (M+2, 3.1), 237 (5.11), 209 (13), 183 (21), 157 (18), 95 (100).

2,6-Diphenyl-8-(4`-fluorophenyl)-9-methyl-pyrazolo[4`,3`:5,6]pyrimido[2,3-c][1,2,4]triazole (6)

A mixture of 14 (0.4g, 1.0 mmol) and benzoic acid hydrazide (0.185g, 1.0 mmol) in DMF (25 ml) refluxed for 2h, cooled. The resulting solid collected by filtration and crystallized from DMF to give compound 6 as pale brown powder. Yield 94%, m.p. 236-238 ºC. Analytical data; Found: C,71.30;H, 3.85; N,19.79 %. Calculated for $C_{25}H_{17}N_6F$ (420); C,71.42; H, 4.04; N, 20.00 %. IR (cm^{-1}) γ: 2840, 2917 (aliph CH), 1584,1617 (C=N), 1489 (deformation of CH_3), 1203(C-F), 763,892 (ph). 1HNMR (ppm) δ: 7.99-8.00, 7,09-7.89 (each d, 2H, ar), 7.90-7.98, 7.86-7.87 (each d,2H, ar), 7.43-7.45, 7.276-7.293, 7.258-7.268 (each m,12H, ar),1.25 (s,3H,CH_3).

8-(4`-Fluorophenyl)-6-(pyridine-4`-yl)-2-phenyl-9-methylpyrazolo[4`,3`:5,6]pyrimido[2,3-c][1,2,4] triazole (7)

A mixture of compound 5 (0.6g, 2.0 mmol) and isonicotinic acid (0.2g, 2.0 mmol) in EtOH (25 ml) refluxed for 5h and cooled. The resulting solid was collected by filtration and crystallized from EtOH to give compound 7 as yellow crystals. Yield 70%, m.p. 169-170 ºC. Analytical data; Found: C,71.30; H, 3.85; N,19.79 % . Calculated for $C_{24}H_{16}N_7F$ (421); C,68.40; H, 3.80; N, 23.22 %. IR (cm-1) γ: 2849, 2918.2955 (aliph CH), 1584,1591, 1616 (C=N),1488(deformation of CH3), 1203 (C-F),790-810 (phenyl). ^{1}HNMR (ppm) δ: 7.99-8.00, 7,09-7.89 (each d, 2H,ar), 7.90-7.98, 7.86-7.87 (each d,2H, ar), 7.43-7.45, 7.276-7.293, 7.258-7.268 (each m,12H, ar), 1.25 (s,3H,CH_3).

N-(Pyrazolo[4,3-d]pyrimidin-3-yl)dithioicformic acid hydrazide (8)

A mixture of 5 (0.6g, 2.0 mmol) and dithioic formic acid hydrazide 20 (0.216g, 2.0 mmol) in EtOH (30 ml) refluxed for 4h, cooled. The resulting solid was collected by filtration and crystallized from EtOH to give compound 8 as yellow crystals. Yield 94%, m.p. 163-164 ºC. Analytical data; Found: C,55.40; H, 3.55; N,20.33; S,15.40 %. Calculated for $C_{19}H_{15}N_6FS_2$ (410); C,55.60; H, 3.65; N, 20.48; S,15.60 %. IR (cm^{-1}) γ: 3300-3100 (b, NH), 2919 (aliph CH), 1584,1615 (C=N), 1489 (deformation of CH3), 1203(C-F), 1180 (C-S), 802,933 (ph). ^{1}HNMR (ppm) δ: 8.5, 9.0 (each s, 2H, NHNH), 7.91-7.92 (each d, 2H,ar), 7.898-7.899, 7.43-7.45 (each m,4H, ar), 7.28-7.29, 7.26-7.27, 7.25-7.26 (each s,3H, ar), 4.8 (s,1H,SH), 1.25(s,3H,CH_3). ^{13}CNMR (ppm) δ: 161 (C=S), 152 (C-S), 138.3 (C-F), 137.58 (C=N), 121.16, 126.62, 128.92 (ar carbons), 109 (C-C), 14.14 (C-CH_3).

Pyrazolo[4`,3`:5,6]pyrimido[2,3-c][1,2,4]triazole-6-thione (**5**)

Compound 8 (0.5g, 1.0mmol) and dry DMF (25 ml) were refluxed for 3h, cooled. The resulting solid was collected by filtration and crystallized from DMF to give compound 5. Yield 94%, m.p. 182-184 ºC.

Synthesis of hydrazo-compound (bis-compound)1,2-Di-(1-phenyl-3-methyl-4-arylpyrazolo-pyrimidin-6-yl)hydrazine (9)

A mixture of compound 2 (0.6g, 2.0 mmol) and compound 2 (0.6g, 2.0 mmol) in isopropyl alcohol (60 ml) refluxed for 6h, cooled and concentrated. The resulting solid collected by filtration and crystallized from ethanol to give compound 9 as yellow crystal. Yield 89%, m.p. 250-252 ºC. Analytical data; Found: C,67.64; H, 3.85; N,21.89 %. Calculated for $C_{36}H_{26}N_{10}F_2$ (636) ; C,67.92; H, 4.08; N, 22.01%. UV(nm) λ_{max}: 434 (ε 1.60). IR (cm-1) γ: 3150 (NH), 2980 (aliph CH), 2352 (aza N-N group), 1528,1590 (C=N), 1450,1498 (deformation of CH3), 1347 (NCN), 1230 (C-F), 810,908 (ph). ^{1}HNMR (ppm) δ: 7.80-7.85 (d,3H,aromatic), 7.53 (s,1H,ar), 7.49-7.51 (d, d, 2H, ar), 7.33, 7.34, 7.35 (each s,3H,ar), 3.41-3.5 (2H, NHNH), 1.25 (s,3H,CH3).

1,2-Diheteroaryl azo compound (10)

A mixture of compound 9 (0.6g, 0.9 mmol) and sulphur powder (0.028g, 0.9 mmol) in dry benzen (35 ml) was refluxed for 4h, cooled. The resulting solid collected by filtration and crystallized from benzene to give compound 10 as yellow powder. Yield 92%, m.p. 245-246 ºC. Analytical data; Found: C,67.90; H, 3.55; N,21.80 % . Calculated for $C_{36}H_{24}N_{10}F_2$ (634); C,68.13; H, 3.78; N, 22.08 %. UV(nm) λ_{max}: 540 (ε, 0.90). IR (cm^{-1}) γ: 2850, 2915 (aliph. CH), 1867 (N=N), 1581,1591 (C=N), 1482,1496 (deformation CH3), 1335 (NCN), 1251 (C-F), 803,906 (ph). ^{1}HNMR (ppm) δ:7.89-7.90, 7.82-7.83 (each d,2H,ar), 7.43-7.48 (m,10H, ar), 7.33-7.35 (d, 2H, ar), 7.25-7.28 (m, 4H, ar), 1.25 (s,1H,CH_3). ^{13}CNMR (ppm) δ: 142 (C-F), 138.30 (C=N), 137 (C-N), 121.13, 121.61, 128.61, 128.65, 128.92, 129.19,129.25 (ar. carbons), 99.64 (C-C), 14.15 (C-CH3). m/s (Int.y.): 636 (M+2, 0.5%), 303 (13), 208 (28), 156 (5.8), 312 (28), 95 (100), 52(5).

5. Biological Activity

The electron withdrawing/ donating nature of heterocyclic nitrogen systems in diamine influences the nucleophility of the amino group [8]. In addition, fused heteropolycyclic nitrogen systems exhibit marked biological and pharmacological effects obtained from ring closure reactions of asymmetric diamines [9-10]. Moreover, presence of fluorine atoms enhances the biological activity. Accordingly, this work is aimed to evaluate the effects of fluorinated heterocyclic systems on the cellobiase activity of some fungi.

The effect of the newly synthesized fluorinated heteropolycyclic systems on the activity of cellobiase produced by *Aspergillus Nidulans* was studied according to the standard method. DMF was used as a solvent and a control, at pH 4.8-5 incubated at 50 °C for 1 hour. The released reducing sugar was estimated calorimetrically at 540 nm as an indicator for the enzyme activity. The results obtained were recorded in Table 1.

Table 1. The effects of fluorinated hetero poly cyclic nitrogen systems on the cellobiase activity of A.N. Fungi Concentration.

Nitrogen	Concentrations			Compound
Percent %	10 µg/ml	100 µg/ml	1000 µg/ml	number
25.1	0.33	0.35	0.37	14
24.41	0.34	0.36	0.38	15
23.26	0.31	0.35	0.36	16
23.27	0.30	0.30	0.35	19
22.01	0.35	0.36	0.41	22
22.08	0.35	0.37	0.45	23

* Blank: 0.35 µg/ml (without substance or DMF).

** DMF: 0.04 µg/ml as solvent.

From the results obtained in table 1 we conclude that the overall activities of the tested compound are 10> 9> 2> 3> 4> 6, the highly activity of compounds 10 and 9 may be due to a higher nitrogen percent and the present of fluorine atoms. Thus the increasing of nitrogen percent in the active systems possibly enhances their activity.

6. Conclusion

This investigation reported a novel synthetic application toward an interesting fluorine substituted fused heterocyclic nitrogen systems via ring closer reactions of hydrazinopyrazolopyrimidine with bifunctional compounds. These synthesis systems were evaluated as enzymatic effects towards cellobiase activity

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