

# Synthesis and Characterization Antifungal Fluorine Substituted Fused Heterobicyclic Nitrogen Systems Containing 1,2,4-triazine Moiety

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Received: April 26, 2018

Accepted: May 1, 2018

Online Published: May 2, 2018

doi:10.5539/jmbr.v8n1p41

URL: <https://doi.org/10.5539/jmbr.v8n1p41>

## Abstract

Synthesis of some new full fluorinated heterobicyclic nitrogen systems containing 1,2,4-triazine moiety (**4-10**) have been deduced from heterocyclization of 6-(2'-trifluoroacetylamino)phenyl-3-(4'-fluorophenyl)-1,2,4-triazin-5-one (**3**) with active oxo / halo-compounds. Structure of the products have been established upon their elemental and spectral data. Most of these systems exhibited a good to moderate antifungal activities.

**Keyword:** Synthesis, full fluorinated heterobicyclic, antifungal

## 1. Introduction

The introduction of fluorine atoms to functionally 1,2,4-triazines improve their physical, chemical and biological, medicinal properties. Most of fused heterobicyclic nitrogen systems containing 1,2,4-triazine moiety exhibit a good biocidal activity which may be formed of biodynamic systems. Thus, the present work tends to synthesize of full fluorinated fused heterobicyclic nitrogen systems starting from 1,2,4-triazine moiety in view of their antifungal activities.

## 2. Chemistry

Due to a highly medicinal and biological activity of fluorinated 3-thioxo-1,2,4-triazin-5-one, the present article use of these systems as starting material. Cyclocondensation of isatin with thiosemicarbazide under refluxed in base-medium produced 6-(2'-aminophenyl)-1,2,4-triazin-5-(2H,4H)one (**1**) which upon refluxed with ethyl trifluoroacetate in THF afforded 6-(2'-trifluoroacetamidophenyl)-1,2,4-triazin-5(2H) one (**2**). A simply nucleophilic attack of compound **2** with 4-fluoroanilin in refluxed ethanol yielded 3-(4'-fluorophenyl)-6-(2'-trifluoroacetamido phenyl)-1,2,4-triazine (**3**) (Scheme 1).

The new targets have been obtained from heterocyclization of compound **3** with activated carbonyl such as diethyl oxalate, (THF) chloro acetic acid (DMF), chloroacetylchloride (DMF) and chloroacetonitrile (DMF) to give the imidazolotriazinons **4-7** (Scheme 2).

Presence of both active methylene and /or amino groups in the structures of **6&7** was confirm from condensation with arylaldehyde (AcOH) to give the arylidene **8** and /or the Schiff base **9** (Scheme 3).

## 3. Experimental

### 3.1 Materials and Methods

Melting points determined on an electrothermal Bibby Stuart SMP<sub>3</sub> (UK) Scientific melting point apparatus and are uncorrected. The infrared (IR) spectra recorded on Perkin-Elmer RXI FT-IR infrared spectrophotometer using the KBr pellet technique. Electronic absorption spectra were recorded in DMF on Shimadzu UV- Visible 3101 PC spectrophotometer. <sup>1</sup>H NMR spectra recorded on a Bruker DPX-400FT-NMR spectrometer at Cairo-University, Egypt. Using tetramethylsilane as the internal standard DMSO-d<sub>6</sub> as a solvent (Chemical shifts in δ, ppm). <sup>19</sup>F-NMR Spectra determined at 84.25 MHz using hexafluorobenzene as a solvent. Splitting patterns were designated as follows: *s*: single; *m*: multiple; Mass spectra measured on a GCMS-Q 1000 Ex spectrometer. Elemental analyses were performed on a 2400 Perkin Elmer Series 2 analyzer and the found values within ± 0.4% of the theoretical values. Follow up of the reaction and checking the homogeneity of the compounds made by TLC

on silica gel- protected aluminum sheets (Type 60 F254, Merck). Compounds **1&2** were prepared according to reported method.

### **3-(4'-Fluorophenylamino)-6-(2-trifluoroacetamidophenyl)-1,2,4-triazinone (3)**

Equimolar mixture of **2** and 4-fluoroaniline in ethanol (50 ml) was refluxed for 6h. cooled then poured onto ice. The solid thus obtained filtered off and crystallized from THF to give **3**, yield 70 %, m.p. 315-317 °C. IR ( $\gamma$ )  $\text{cm}^{-1}$ : 3300-3100 (b, NH,NH), 3010 (aromatic CH), 1690 (C=O triazinone), 1630 (C=O, amide), 1230 (C-F).  $^1\text{HNMR}$  ( $\delta$ ) ppm: 13.42, 12.61, 10.88 (each s, 3NH), 7.9-7.25, 7.09-6.55 (each m, 8H of aromatic). M/Z: (int. %) 395 ( $\text{M}^{+2}$ , 1.5 %), 96 (100). Analysis Calcd for  $\text{C}_{17}\text{H}_{11}\text{N}_5\text{F}_4\text{O}_2$  (393), C, 51.9; H, 2.79; N,17.81; F,19.33. Found: C. 51.70; H,2.55; N.17.65; F,18.95 %.

### **1-(4'-Fluorophenyl)-6-(2'-trifluoroacetamidophenyl)-imidazolo[3,2-b][1,2,4]triazin-2,3,7-trione (4)**

A mixture of **3** (0.01mol) and diethyl oxalate (0.01 mol) in dioxan (50 ml) was refluxed for 4 h, cooled .The solid produced filtered off and crystallized from THF to give **4**, yield 65 %, m.p. 330-332 °C. IR ( $\gamma$ ) $\text{cm}^{-1}$ : 3200 (NH), 1680, 1660 (2 C=O), 1250(C-F).  $^1\text{HNMR}$  ( $\delta$ ) ppm: 13.44 (s, NH), 7.92-7.25, 7.11-6.60 (each m, 8H of aromatic). Anal. Calcd for  $\text{C}_{19}\text{H}_9\text{N}_5\text{F}_4\text{O}_4$  (447), C,51.0; H, 2.01; N,15.65; F,17.0. Found: C,50.70; H,1.89; N,15.34; F,16.68 %.

### **1-(4'-Fluorophenyl)-2,3-dihydro-6-(2'-trifluoroacetamidophenyl)-imidazolo[3,2-b][1,2,4]triazin-3,7-dione (5)**

A mixture of **3** (0.01mol) and monochloroacetic acid (0.01 mol) in DMF (20 ml) was refluxed for 4h, cooled then poured into ice. The yielded solid filtered off and crystallized from EtOH to give **5**, yield 68% , m.p. 225-227 °C. IR ( $\gamma$ )  $\text{cm}^{-1}$  : 3080 (NH), 2850 (aliphatic  $\text{CH}_2$ ), 1660 (C=O), 1480 (deformation  $\text{CH}_2$ ), 1230 (C-F), 880,820 (aromatic ring).  $^1\text{HNMR}$  ( $\delta$ ) ppm: 12.31 (NHCO), 8.29-7.41, 7.39-7.29 (each m, 8H of aromatic), 5.01 (m, N-CO- $\text{CH}_2$ ). Anal. Calcd for  $\text{C}_{19}\text{H}_{11}\text{N}_5\text{F}_4\text{O}_3$  (533), C, 42.77; H, 2.06; N,13.13; F,14.25. Found: C,42.50; H,1.88; N,12.98; F, 14.05 %.

### **1-(4'-Fluorophenyl)-2,3-dihydro-6-(2'-trifluoroacetamidophenyl)-imidazolo [3,2-b][1,2,4] triazin-2,7-dione (6)**

Chloroacetyl chlorid (0.01mol) was added to a solution of **3** (0.01 mol) in DMF (20 ml) and refluxed for 2 h, cooled then poured into ice. The solid obtained filtered off and crystallized from MeOH to give **6**, yield 60 %, m.p. 260-262 °C. IR ( $\gamma$ )  $\text{cm}^{-1}$  : 3100 (NH), 2900 (aliphatic  $\text{CH}_2$ ) 1700, 1670 (2C=O), 1440 (deformation  $\text{CH}_2$ ), 1230 (C-F), 850,830 (aromatic ring).  $^1\text{HNMR}$  ( $\delta$ ) ppm: 13.0 (NH), 10.0 (s, 1H, OH of imidazole ), 7.92-7.52, 7.47-7.13 (each m, 8H of aromatic), 4.0 (s, 2H,  $\text{CH}_2$  protons). Anal. Calcd for  $\text{C}_{19}\text{H}_{11}\text{N}_5\text{F}_4\text{O}_3$  (533), C, 42.77; H, 2.06; N, 13.13; F, 14.25. Found: C. 42.60; H, 1.85; N, 13.00; F, 13.85 %.

### **1-(4'-Fluorophenyl)-6-(2'-trifluoroacetamidophenyl)-3-aminoimidazolo[3,2-b][1,2,4] triazin-7-one (7)**

Equimolar amounts of **3** and Chloroacetonitrile in DMF (20 ml) was refluxed for 3h, cooled then poured into ice. The solid obtained filtered off and crystallized from dioxin to give **7**, yield 55 %, m.p. 288-290 °C. IR ( $\gamma$ )  $\text{cm}^{-1}$  : 3300 ( $\text{NH}_2$ ), 1680 (C=O), 1250 (C-F), 890, 820 (aromatic ring).  $^1\text{HNMR}$  ( $\delta$ ) ppm: 12.5 (s, NH); 8.38 (s,1H, CH= of imidazole), 7.92-7.63, 7.52-7.19 (each m, 8H of aromatic), 3.64 (s, 2H,  $\text{NH}_2$ ). MS (ref. int.%) 434( $\text{M}^{+2}$ , 88%), 96(100,  $\text{C}_6\text{H}_4\text{F}$ ). Anal. Calcd for  $\text{C}_{19}\text{H}_{12}\text{N}_6\text{F}_4\text{O}_2$  (432), C, 52.77; H, 2.77; N, 19.44; F, 17.59. Found: C. 52.55; H, 2.61; N, 19.03; F, 17.39 %.

### **The arylidene derivative 8**

A mixture of compound **5** ( 0,01 mol) and 4- chlorobenzaldehyde (0.01mol) in glacial acetic acid (20 ml) was refluxed for 2h, cooled then poured into ice. The solid thus obtained filtered off and crystallized from AcOH to give **8**, yield 66 %, m.p. 248-250 °C. IR ( $\gamma$ )  $\text{cm}^{-1}$  : 3080 (NH), 1700, 1680 (2C=O), 1230 (C-F), 890, 810, 790 (aromatic ring), 700 (C-Cl).  $^1\text{HNMR}$  ( $\delta$ ) ppm: 13.60 (s, 1H, NH); 9.94 (exocyclic  $\text{CH}=\text{C}$ ), 7.90-7.61, 7.47-7.36, 7.18-6.55 (each m, 12H, aromatic protons). Anal. Calcd for  $\text{C}_{27}\text{H}_{13}\text{N}_5\text{F}_4\text{ClO}_3$  (567), C, 57.14; H, 2.29; N, 12.34; F, 13.40; Cl, 6.34. Found: C. 56.90; H, 2.11; N, 12.12; F, 13.15; Cl, 6.12 %.

### **The Schiff base 9**

A mixture of **7** (0.01 mol) and 4-chlorobenzaldehyde (0.01 mol) in glacial acetic acid (20 ml) was refluxed for 1h, cooled then poured into ice. The yielded solid filtered off and crystallized from EtOH to give **9**, yield 66 %, m.p. 278-280 °C. IR ( $\gamma$ )  $\text{cm}^{-1}$ : 3150 (NH), 1680 (C=O), 1590 (C=N) 1230 (C-F), 900, 880, 810 (aromatic ring), 710 (C-Cl).  $^1\text{HNMR}$  ( $\delta$ ) ppm: 13.0 (1H, NH); 10.01 (s, 1H,  $\text{CH}=\text{N}$ ), 8.8 (s, 1H,  $\text{CH}=\text{C}$ ); 7.96-7.94, 7.71-7.69, 7.59-7.00 (each m, 12H, aromatic protons). Anal. Calcd for  $\text{C}_{26}\text{H}_{15}\text{N}_6\text{F}_4\text{ClO}_2$  (555), C, 56.21; H, 2.70; N, 15.13; F,13.69; Cl,6.48. Found: C.55.91; H, 2.55; N, 15.01; F, 13.51; Cl,6.19 %

### 1-(4'-Fluorophenyl)-hexahydro-7-(2'-trifluoroacetamidophenyl)-pyrimido[3,2-b][1,2,4] triazin-2,4,8-trione (10)

Equimolar ratio of **3** and diethylmalonate in reflux with THF for 6h, cooled. The solid obtained filtered off and crystallized from dioxin to give **10**, yield 70 %, m.p. 188-190 °C. IR ( $\gamma$ )  $\text{cm}^{-1}$ : 3400-3300 (b, OH-NH), 1700, 1660, 1620, 1590 (C=O), 1480 (deformation  $\text{CH}_2$ ), 1250 (C-F), 820, 770 (aromatic ring), 690 (C-F).  $^1\text{H}$ NMR ( $\delta$ ) ppm: 13.42 (s, 1H NH); 9.621(OH), 7.7-7.4, 7.37-6.59 (each m, 8H of aromatic), 3.67, 3.36 (m, 2H,  $\text{CH}_2$ ). M/Z: (Int. %) 480 ( $\text{M}^+\text{H}_2\text{O}$ , 6.13%), 96 (100). Anal. Calcd for  $\text{C}_{20}\text{H}_{11}\text{N}_5\text{F}_4\text{O}_4$  (461), C, 52.06; H, 2.43; N, 15.52; F, 15.18. Found: C, 51.86; H, 2.22; N, 15.30; F, 15.00 %.

## 4. Results and Discussion

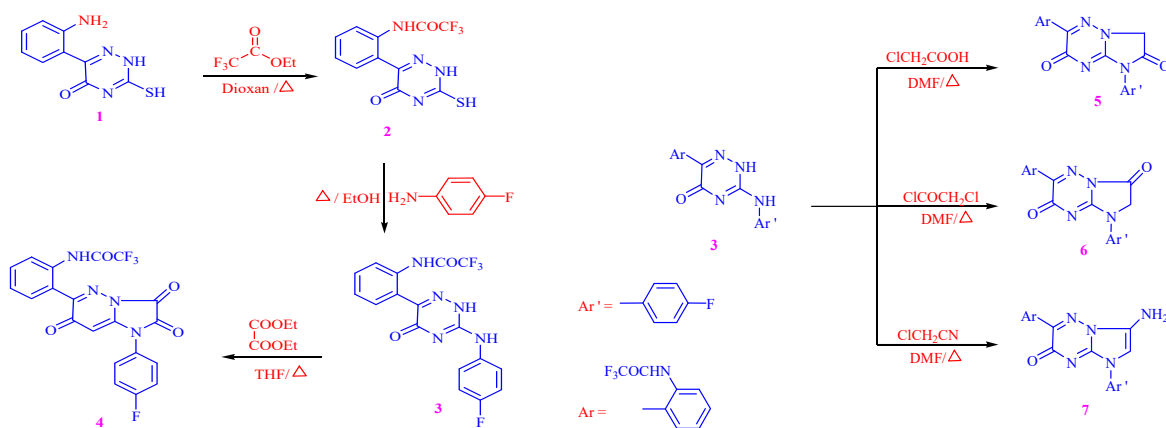
IR spectra of all the synthesized compounds recorded a true-carbonyl groups at  $\sim 1700 \text{ cm}^{-1}$  with other  $\gamma$  at  $1250 \text{ cm}^{-1}$  for C-F, while that of **3** showed a lack's of C=S group. IR spectra of compounds **4-7** exhibited a second pseudo C=O groups attribute to C=O & C-OH group at  $\gamma$   $3500\sim 3400 \text{ cm}^{-1}$ .

Mass fragmentation pattern of compound **4** as shown in Figure 1. Presence of OH groups in the compounds **5&6** is due to adjacent of  $\text{CH}_2$  to C=O. Former structure of **5&6** is more stabilized skeleton due to the enolization formed towards aryl groups.

$^1\text{H}$ NMR spectra of all the new synthesized compounds showed a different resonated signals of aromatic proton at  $\delta$   $8\sim 6.8$  ppm with a peak at  $\delta$   $6\sim 5$  ppm attribute to  $\text{CH}_2$  protons of compounds **5&6**. On other hand,  $^1\text{H}$ NMR spectra these compounds **8&9** recorded the presence of exocyclic CH= protons at  $\delta$   $9.8\& 8.5$  ppm with lack's of  $\text{CH}_2$  protons. It is interest that the interaction between compound **3** and chloroacetonitrile in reflux DMF via elimination of Cl ions followed by cycloaddition of NH (triazine) to  $\text{C}\equiv\text{N}$  group yielded the 3-amino-1-(4'-fluorophenyl)-6-(2'-trifluoroacetamidophenyl)-imidazole[2,3-b]-1,2,4-triazin-7-one (**7**).

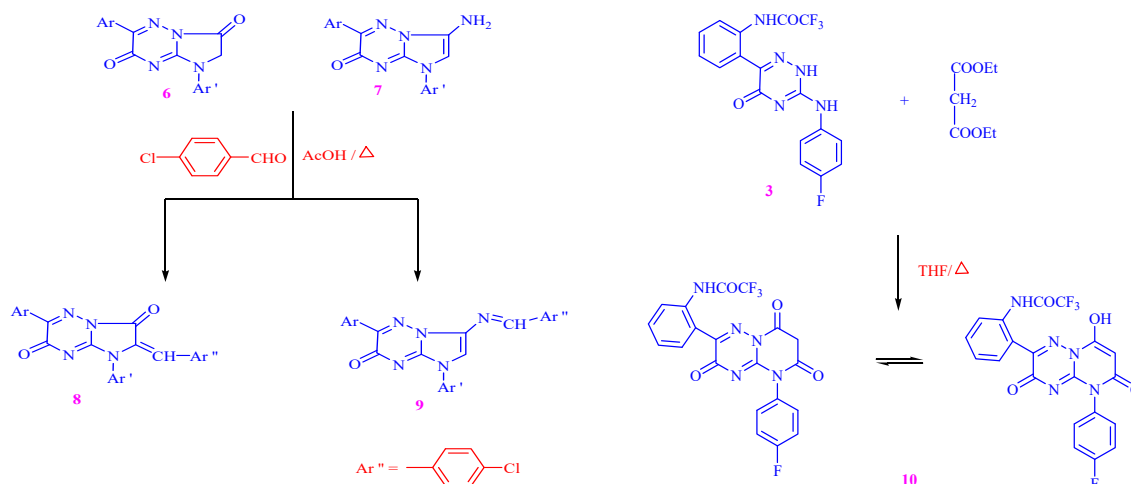
IR of **7** showed the presence of  $\text{NH}_2$  group with as lacks of  $\text{C}\equiv\text{N}$  group.  $^1\text{H}$ NMR spectra give us a supported the structure by recorded the presence of CH= (imidazole) at  $\delta$   $8.38$  ppm in addition to both NH,  $\text{NH}_2$  and aryl protons at  $\delta$   $12.5$ ,  $3.6$  and  $7.7\sim 6.8$  ppm, respective. Formation of compound **7** may be as shown in the Figure 3.

Former structure of compound **10** was deduced from both correct elemental analysis and that spectral measurements. Thus, IR spectrum showed  $\gamma$  at  $3400\sim 3300 \text{ cm}^{-1}$  attribute to NH - OH functional groups, in addition at  $\gamma$   $1700$ ,  $1660$ ,  $1620\sim 1590 \text{ cm}^{-1}$  for  $2\text{C}=\text{O}$  and CO-NH groups with  $\gamma$  at  $1480$  and  $1250 \text{ cm}^{-1}$  to aliphatic and C-F groups.  $^1\text{H}$ NMR spectrum of **10** recorded the resonated signal at  $\delta$   $13.42$ ,  $9.62$ ,  $7.7\sim 7.4$ ,  $7.37\sim 6.59$  ppm for NH, OH, aromatic protons with  $\delta$  at  $3.67\sim 3.36$  ppm attribute to  $\text{CH}_2$  protons. Mass fragmentation pattern recorded the molecular ion peak with introduction of one mole of  $\text{H}_2\text{O}$  with a base peak at 96 (Figure 2).



Scheme 1

Scheme 2



Scheme 3

Scheme 4

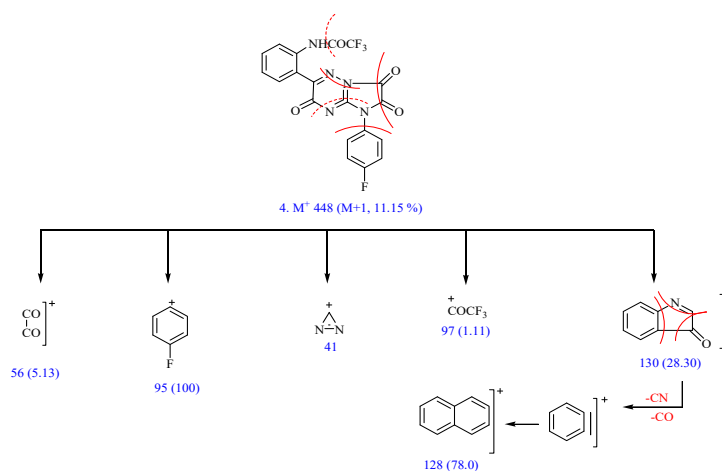


Figure 1. Mass Fragmentation Pattern of compound 4

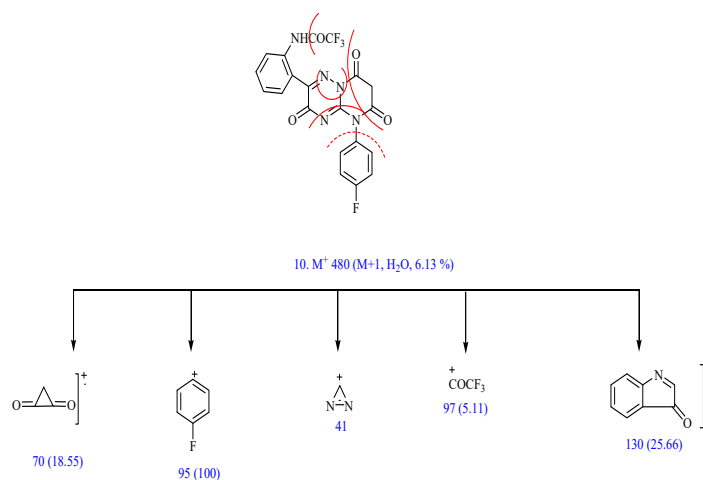


Figure 2. Mass Fragmentation Pattern of compound 10

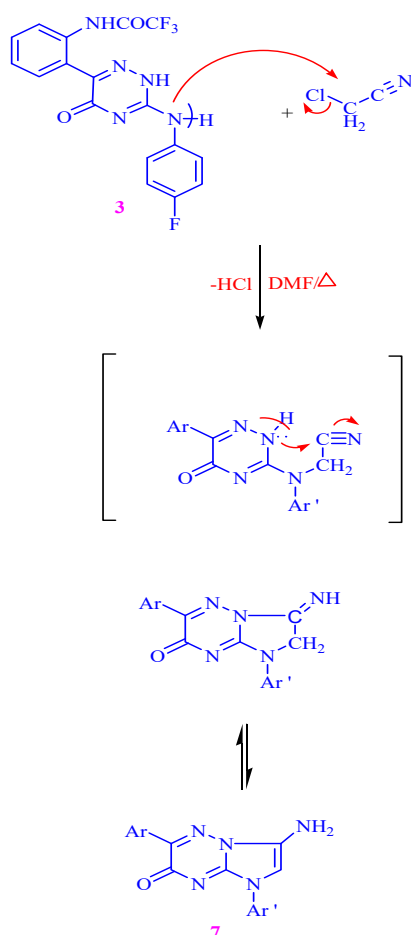


Figure 3. A possible formation of 7 from 3

## 5. Antifungal Activity

One from the important roles of 1,2,4-triazinones is an enzymatic (cellobiase) effect is on the fungi. Thus, the new synthesized full fluorinated fused heterobicyclic nitrogen systems containing 1,2,4- triazinone moiety have been evaluated as cellobiase agents by used the method described<sup>[15]</sup>. DMF used as solvent and control towards the selected fungi, *Aspergillus nidulans*, then added the mixture of enzyme solution with the substrate (cellobiase) dissolved in citrate phosphate buffer at pH 4.8 and incubated at 50 °C for one hour. The related reducing sugar was estimated colorimetrically at 540 nm as an indication for the enzyme activity. 3,5-Dinitrosalicylic acid (DNS) is used in colorimetric determination of reducing sugars and to analyze glycosidase (glycoside hydrolase) activity by quantitation of enzymatically released reducing sugar. The results obtained recorded in the table 1. We can be conclude that increasing the reducing sugar in the case of compounds 3 & 7 at high concentration, and in the case of compounds 4 at low concentration is due to the presence of biodynamic systems with CF<sub>3</sub> & CF.

Table 1. Effect on cellobiase activity produced by *A. spergillus nidulans*

Compound No	10 Mg / ml	100 Mg / ml	1000 Mg / ml
3	0.35	0.35	0.41
4	0.35	0.35	0.17
5	0.33	0.33	0.34
6	0.32	0.36	0.35
7	0.42	0.35	0.35
10	0.44	0.38	0.40

Blank 0.35 Mg / ml; DMF: 0,04 Mg / m

## 6. Conclusion

The novel full fluorinated imidazole /pyrimido-1,2,4-triazinones were synthesized and evaluated as cellobiase activity by use *Aspergillus nidulans* fungi. It has been an important research organism for studying eukaryotic cell biology for over 50 years, being used to study a wide range of subjects including recombination, DNA repair, mutation, cell cycle control, tubulin, chromatin, nucleokinesis, pathogenesis, metabolism, and experimental evolution. were the compounds **3,7&10** exhibit a large effect towards a reducing sugar.

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