MANSOURA UNIVERSITY
FACULTY OF PHARMACY

Imidazo[2',1':2,3]thiazolo[4,5-*d*]pyridazin Analogues as New Scaffold of DHFR Inhibitors

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http://scholar.google.com.eg/citations?user=8Vsp-IAAAAAJ&hl=en&oi=ao

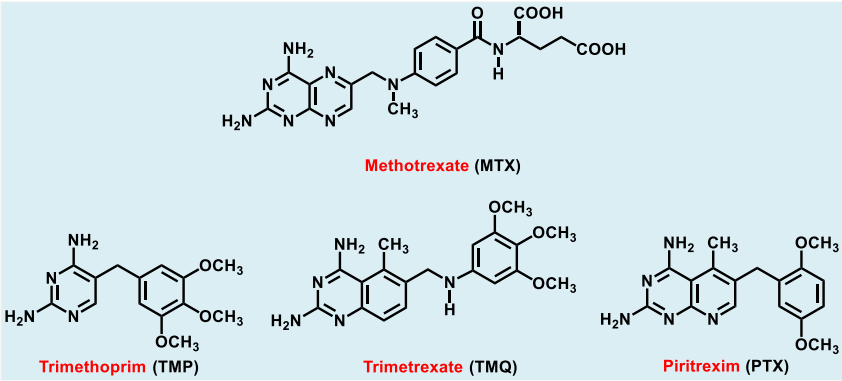
Introduction

- DHFR an enzyme plays an important role in human physiology. Blocking of its activity is a key step in treating :
  - Cancer,
  - AIDS related infections, and
  - Bacterial or parasitic infections.[1-2]
- Many useful drugs have been developed to date, however, the issue of drug toxicity, and drug resistance make it imperative that new inhibitors of DHFR with increased selectivity and lower toxicity be designed.

1. Masur, H.; Kaplan, J.E.; Holmes, K.K. *Ann. Intern. Med.* 2002, *137*, 435-478.
2. Sparano, J.; Sara, C. *Curr. Opin. Oncol.* 1996, *8*, 392–399.

2

Dihydrofolate Reductase Inhibitors



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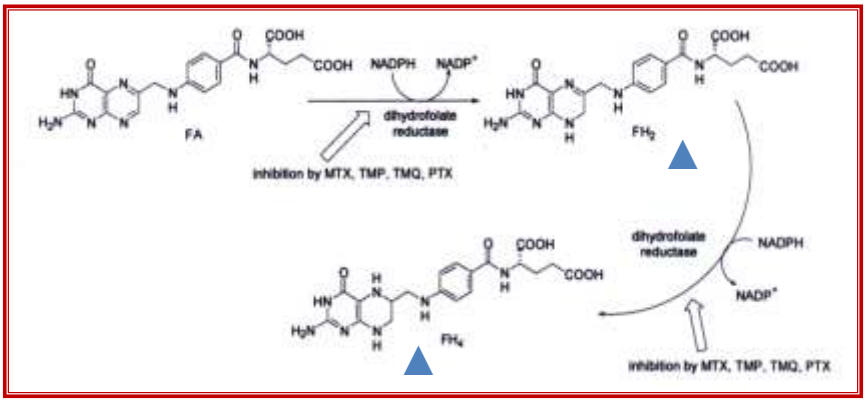
Sites of Action of Antifolates



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DHFR Inhibitors in Treatment of Cancer

DHFR plays a critical role in regulating the amount of FH_2 in the cell which is important for cell growth and proliferation

Inhibitors of DHFR act as antimetabolites blocked the formation of FH_4 leading to DNA synthesis inhibition and hence prevent cell division.

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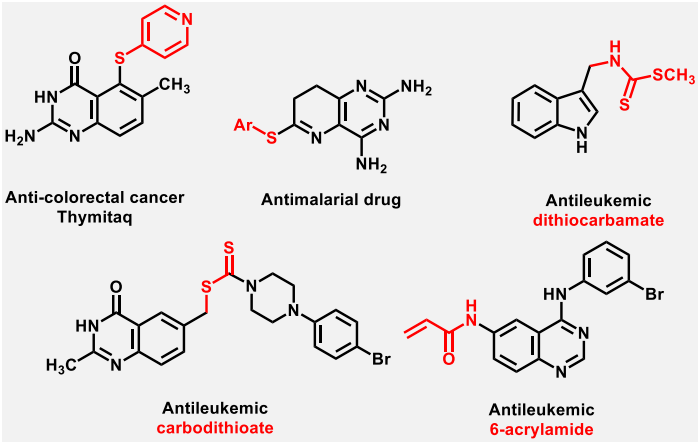
Literature Lead Compounds



1. Bavetsias, V.; Marriott, J.H.; Melin, C.; Kimbell, R.; Matusiak, Z.S.; Boyle, F.T.; Jackman, A.L. *J. Med. Chem.* 2000, 43, 1910-1926.
2. Sheng-Li, C.; Yu-Ping, F.; Yu-Yang, J.; Shi-Ying, L.; Guo-Yu, D.; Run-tao, L. *Bioorg. & Med. Chem. Lett.* 2005, 15, 1915-1917.
3. Wyss, P.C.; Gerber, P.; Hartman, P.G.; Hubschwerlen, C.; Locher, H.; Marty, H.; Stahl, M. *J. Med. Chem.* 2003, 46, 304-2312.

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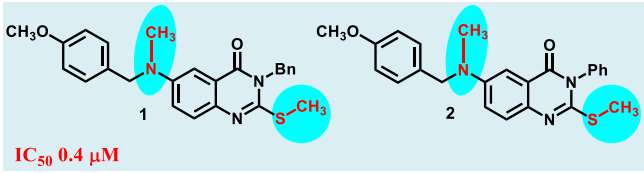
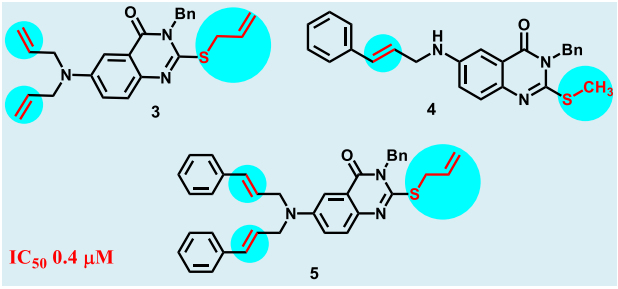
Results of Some Previous Efforts

Part 1:



H I. El-Subbagh, Bioorg. & Med. Chem. 14 (2006) 8608-8621.

Part 2:



H. I. El-Subbagh. Bioorg. & Med. Chem. 18 (2010) 2849-2863.

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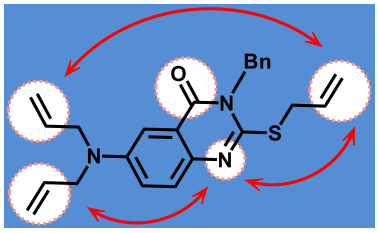
Parts 1 & 2 concluded that :

- The following pharmacophoric features proved to be critical for biological activity:
  - The 4-carbonyl fragment,
  - The basic nitrogen atom at N-1,
  - The hydrophobic p-system regions,
  - The relative spatial distances of those groups.
  - Recognition with key amino acid Arg38 and Lys31 are essential for binding and biological activities.



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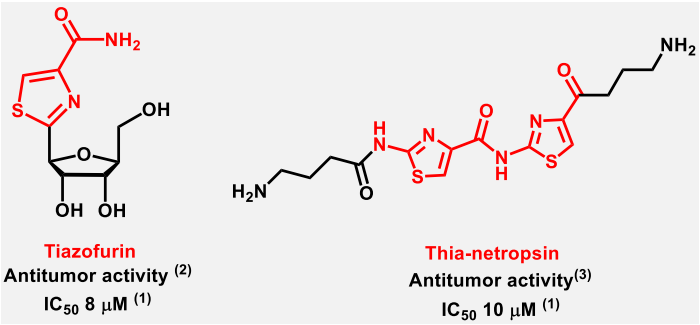
More Literature Lead Compounds



1. Taniki, T., Prajda, N., Monden, Y., & Weber, G. Cancer biochemistry biophysics, 1993, 13(4), 295-302.
2. Pućkowska, A., Midura-Nowaczek, K., & Bruzgo, I. Acta poloniae pharmaceutica, 2008, 65(2), 213-215.
3. Gahtori P, Ghosh SK, Parida P, Prakash A, Gogoi K, Bhat HR, Singh UP. Experimental parasitology, 2012; 130(3):292-9

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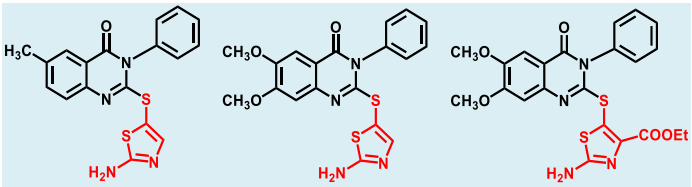
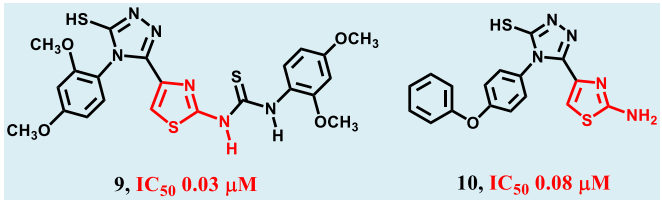
Part 3:



H.I. El-Subbagh. Eur. J. Med. Chem., 2013, 63, 33-45.

Part 4:



H.I. El-Subbagh. Eur. J. Med. Chem., 2013, 66, 135-145.

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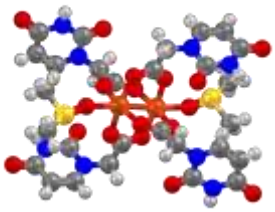
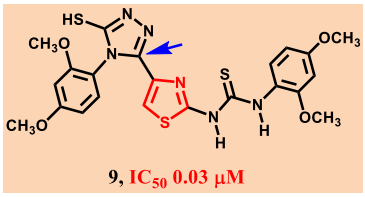
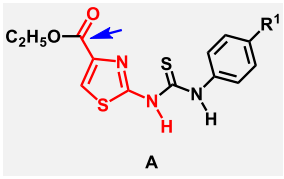


Rational Design

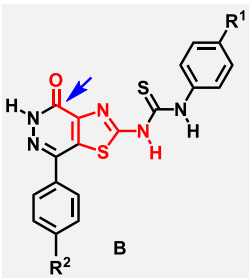
❖ **Bioisosteric modification of the lead 9 using the ester precursors (A) to get the cyclized thiazolo[4,5-*d*]pyridazin-4(5H)-one derivatives (B)**



9, IC_{50} 0.03 μM

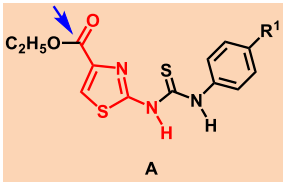


A

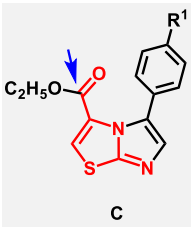


B

- Replacement of thiazole scaffold in A with imidazo[2,1-*b*]thiazoles (C) and its use to get the cyclized imidazo[2',1':2,3]thiazolo[4,5-d]pyridazin (D)



A



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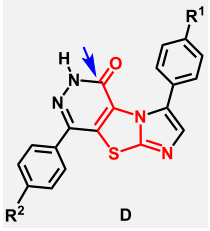
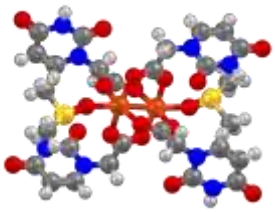
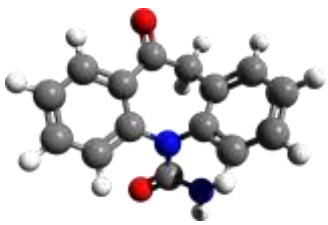


D

13



Synthetic Routes



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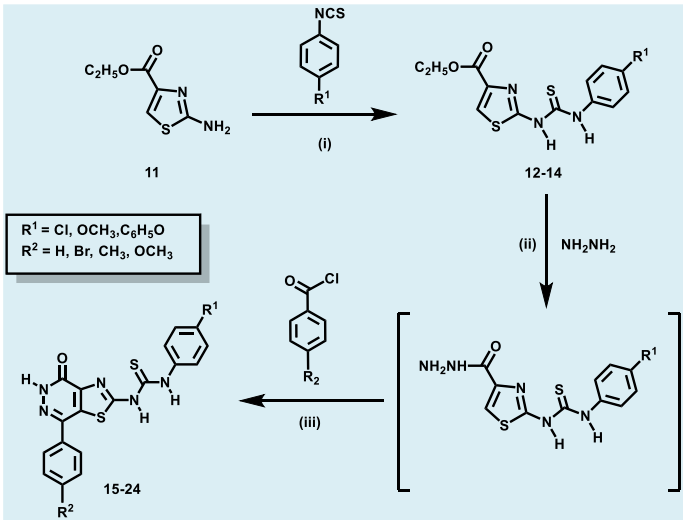
Scheme 1



Reagents & Conditions:
(i) EtOH, 80°C, 20 h.; (ii): NH_2NH_2, $CHCl_3$, r.t., 24 h.; (iii) Benzoyl chloride derivatives, pyridine, r.t., 24 h.

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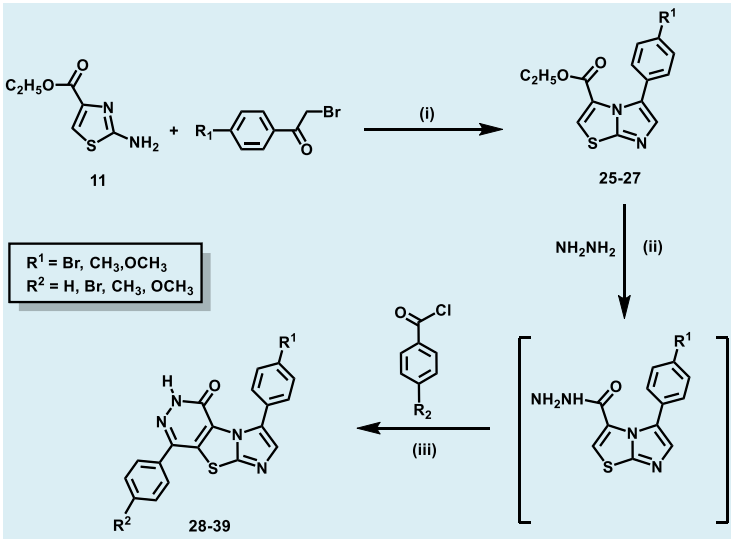
Scheme 2



Reagents & Conditions
(i) $(CH_3)_2CO$, 60°C, 24 h.; (ii) NH_2NH_2, $CHCl_3$, 60°C, 18 h.; (iii) Benzoyl chloride derivatives, pyridine, r.t., 24 h.

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Biological Evaluation

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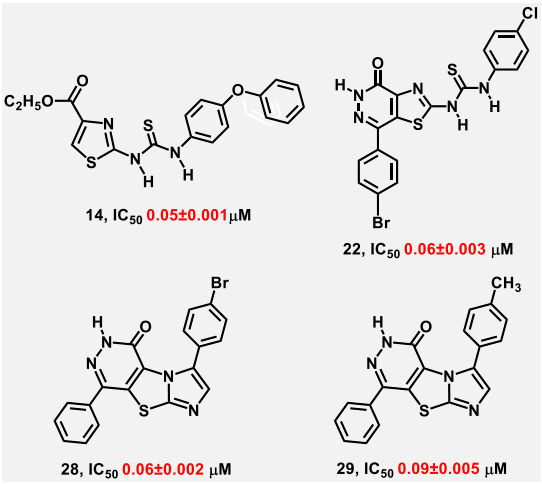
1. DHFR Inhibition

- Compounds were evaluated as inhibitors of bovine liver DHFR.[1,3]
- Results were reported as IC_{50} values.
- Methotrexate was used as a positive control (IC_{50} 0.08 μM).



1. Adamson, P. C. Journal of clinical oncology, 10, 1359-1364 (1992).
2. Falk, L. C. Clinical chemistry, 22, 785-788(1976).
3. El-Subbagh, H. I. Archiv der Pharmazie, 330, 277-284(1997).

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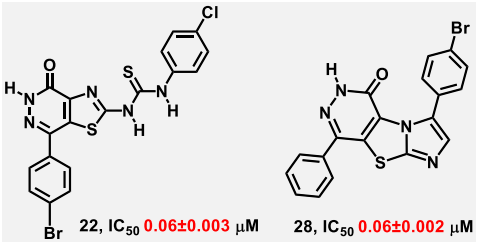
2. Antitumor Screening

- The newly synthesized compounds were subjected to the National Cancer Institute (NCI) *in vitro* disease-oriented human cells screening panel assay for antitumor activity.
- All of the synthesized compounds showed broad spectrum potency toward several tumor cell lines with GI values up to 100%.
- Compound 22 proved lethal to HS 578T Breast cancer cell line while compound 28 proved lethal to OVCAR-3 Ovarian cancer and MDA-MB-435 Melanoma



22, IC_{50} 0.06±0.003 μM 28, IC_{50} 0.06±0.002 μM

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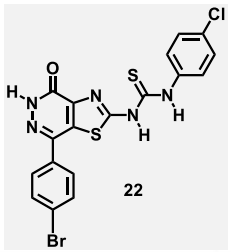
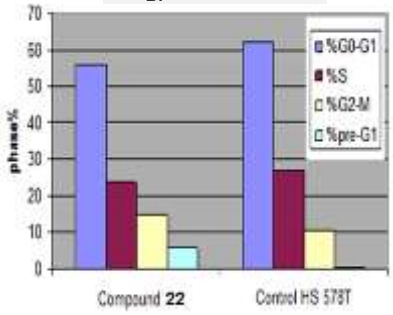
Cytotoxicity MTT Assay for Compound 22

- Anticancer drugs induce cytotoxicity via activation of signal pathways for apoptosis.
- MTT cell viability assay were performed using the mutant HS 578T and 22 which exhibited cytotoxicity with IC_{50} value of 0.8 μM.
- 22 treated cells showed a typical apoptosis pattern of DNA content that reflected G0/G1-, S- ,G2/M phases of the cell cycle, together with a pre-G1 phase related to apoptotic cells.



22



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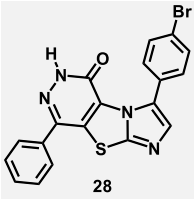
Cytotoxicity MTT Assay for Compound 28



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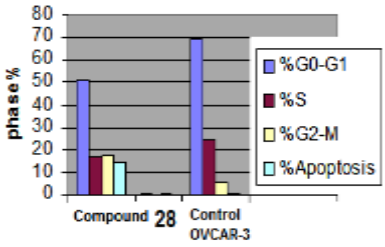
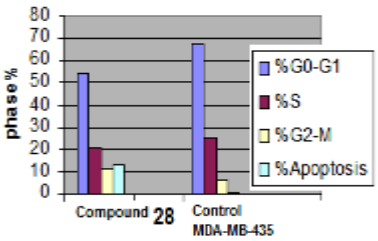
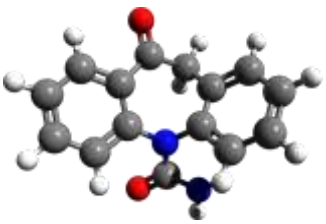




- Compound **28** exhibited cytotoxicity against OVCAR-3 and MDA-MB-435 with IC_{50} value of **0.32, 0.46 µM**, respectively.
- **28** treated cells showed a typical apoptosis pattern of DNA content that reflected G0/G1-, S- ,G2/M phases of the cell cycle, together with a pre-G1 phase corresponding to apoptotic cells.

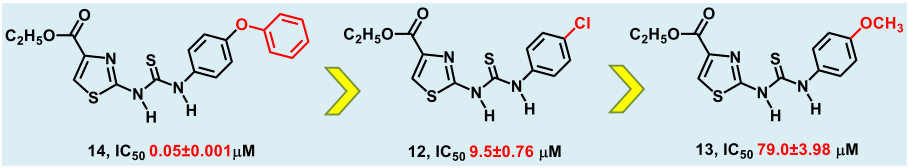
21



Structure Activity Correlation

22

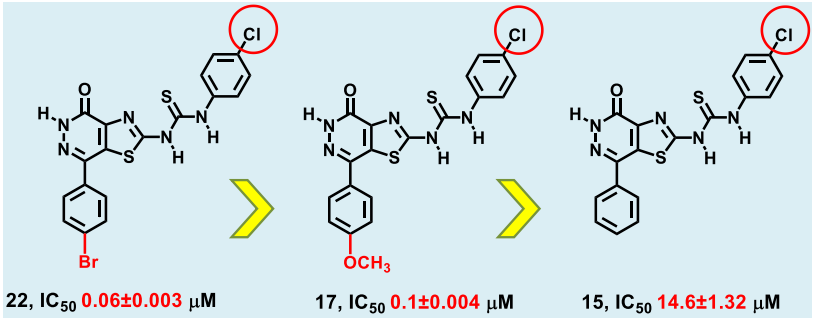
Ethyl 2-[3-(4-substituted-phenyl)thioureido]thiazole-4-carboxylates



- Compound 14 1000 fold more active than 13
- Compound 12 8 fold more active than 13
- Electron withdrawing functions favor the DHFR inhibition potency rather than the electron donating.

23

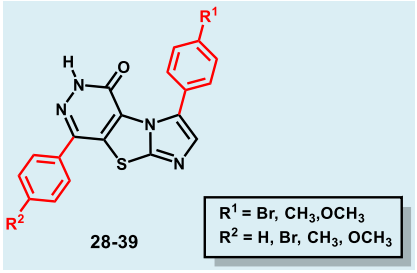
Thiazolo[4,5-*d*]pyridazin-4(5*H*)-one Analogues



- Compound 22 200 fold more active than 15
- Compound 17 140 fold more active than 15
- 7- Substituted phenyl favor the DHFR inhibition potency.

24

Imidazo[2',1':2,3]thiazolo[4,5-*d*]-pyridazin-5(6*H*)-one Analogues



28-39

- The electronegativity at positions 3- and 8- plays a crucial role controlling the DHFR inhibition activity.
- At position 3- the order of activity was
  4-BrPh > 4-CH_3Ph > 4-CH_3OPh.
- At position 8- the order of activity was
  Ph > 4-CH_3Ph > 4-BrPh in most cases.

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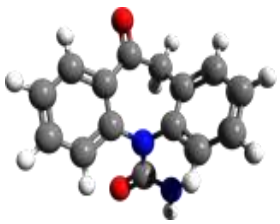
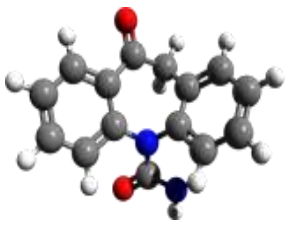


Molecular Modeling Study



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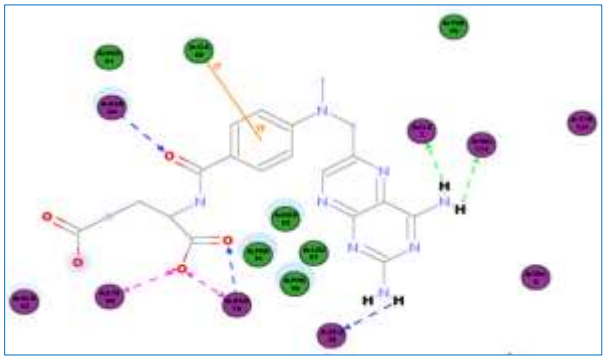
2D Binding Mode in the hDHFR Binding Pocket

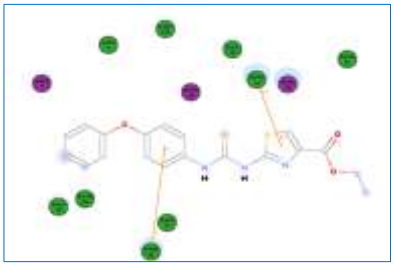
- **MTX** forms Hydrogen bonds with **Asn64, Arg70, Lys68, Glu30, Ile7, Val115** and π-interaction with **Ile60**
- The amino acids **Phe31** and **Arg22** are not one of the key residues involved in the recognition



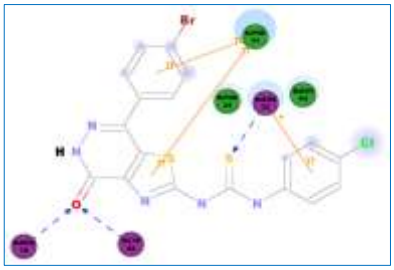
MTX (IC_{50} 0.08 μM)

27

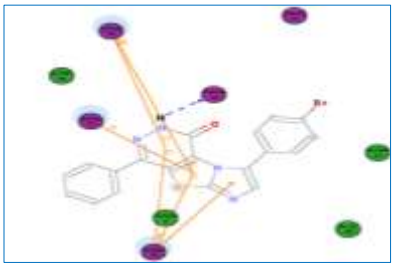
- The active compounds binds to **Phe31** residue linked to the thiazole rings for **22** and **28.**
- **Arg22** residue linked to phenoxy moiety for **14,** 7-Phenyl moiety for **22** and the thiazole ring for **28**
- in addition to a network of π–π interaction and hydrogen bonds



14 (IC_{50} 0.05 μM)

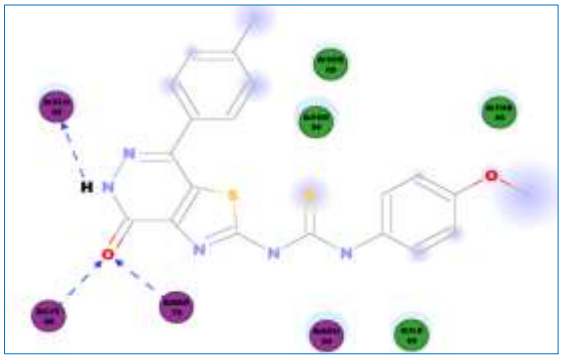


22 (IC_{50} 0.06 μM)



28 (IC_{50} 0.06 μM)

28



The least active **20** (IC_{50} >100 μM)

- This pattern of binding explains the diminished activity of **20** which lack any type of binding with those amino acids.
- The inactive molecules **20** were much more constrained and having different structural attributes related to the aromatic ring.

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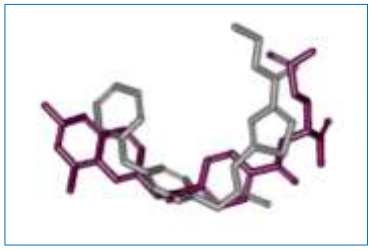
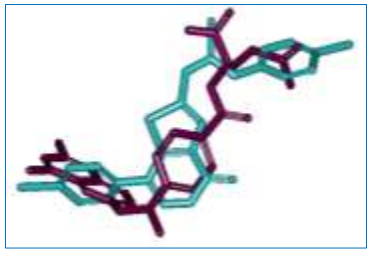
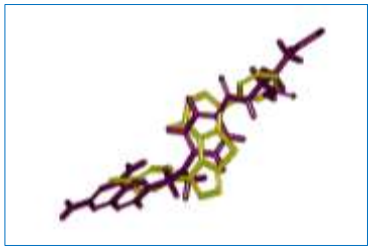
Flexible Alignment



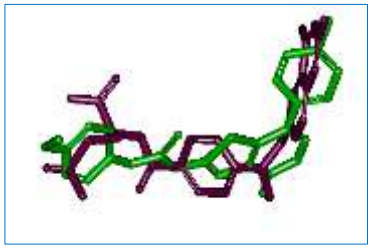
MTX (violet) and **14** (grey)



MTX (violet) and **22** (Cyan)



MTX (violet) and **28** (mustard)

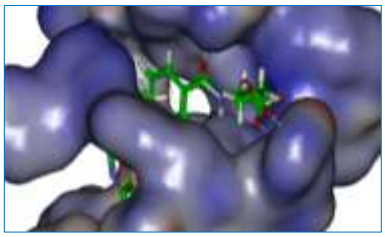


MTX (violet) and **20** (Green)

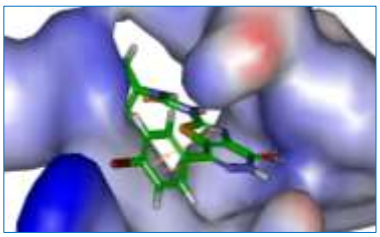
30

Hydrophobic Surface Map

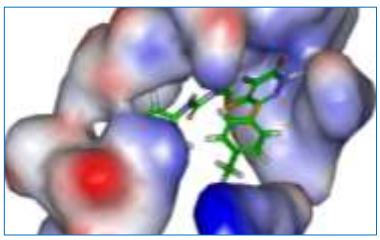
- **22** showed large blue hydrophobic areas responsible for the interaction with the amino acid residues.
- Compound **20** structure was pointed out toward the surface wall of the active site and deprived of any receptor exposure clashes explaining its poor DHFR inhibitory activity.



MTX



The most active **22**



The least active **20**

Blue: hydrogen bonds donor, red: hydrogen bonds acceptor and grey: Hydrophobic moiety.

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Conclusion

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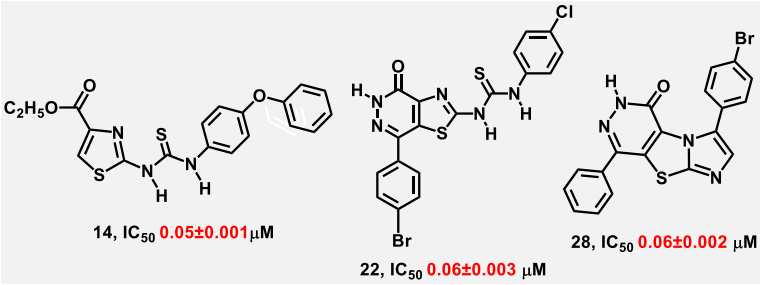
- The study of this new series of

  Thiazolo[4,5-*d*]pyridazin and

  Imidazo[2',1':2,3]thiazolo[4,5-*d*]pyridazin

  as scaffold for DHFR inhibition allowed the allocation of **14**, **22** and **28** as the most active inhibitors (merely comparable to MTX, IC_{50} 0.08 μM).



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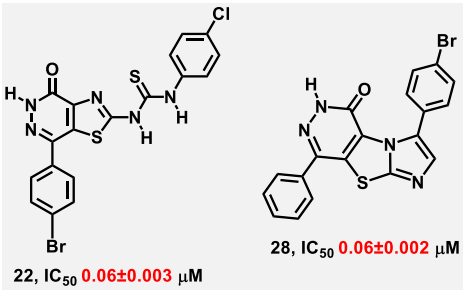
- Compound **22** proved lethal to **HS 578T** breast cancer

  compound **28** proved lethal to **OVCAR-3** Ovarian cancer and

  MDA-MB-435 Melanoma.



- The obtained model could be useful for the development of new class of DHFR inhibitors.

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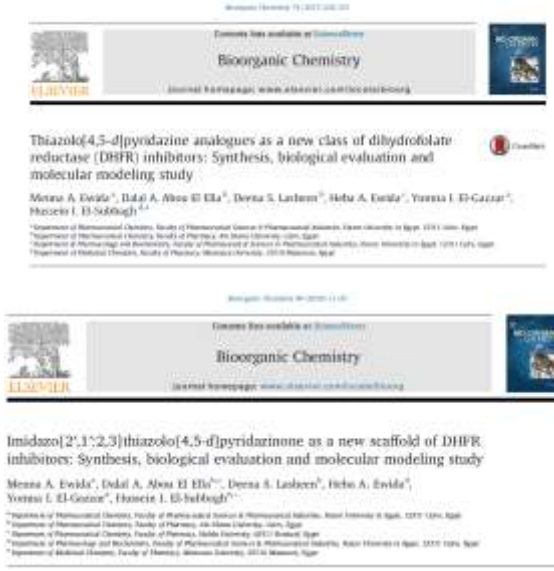
- **The obtained results allowed the publication of two articles:**



Bioorganic Chemistry

Thiazolo[4,5-d]pyridazine analogues as a new class of dihydrofolate reductase (DHFR) inhibitors: Synthesis, biological evaluation and molecular modeling study

Bioorganic Chemistry

Imidazo[2',1':2,3]thiazolo[4,5-d]pyridazinone as a new scaffold of DHFR inhibitors: Synthesis, biological evaluation and molecular modeling study

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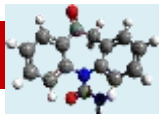
Acknowledgments



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