IMMUNOMODULATORY ACTIVITY OF BENZNIDAZOLE (BZN) IN EHRLICH ASCITES CARCINOMA (EAC) IN SILICO AND IN VIVO

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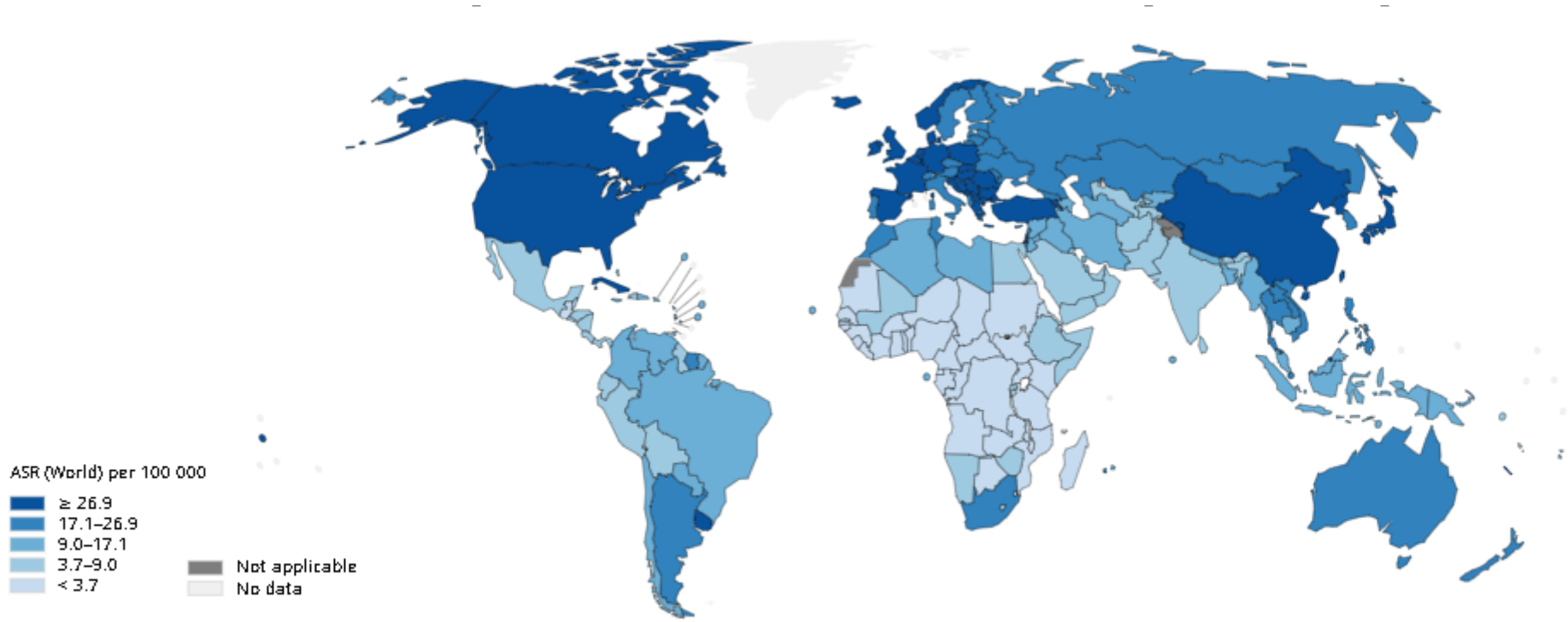
Cancer

Cancer is a major global public health problem represented by around 10 million people killed in 2020 (WORLD HEALTH ORGANIZATION, 2023). Female breast cancer is the most prevalent in the world with (2.6 million) new cases, followed by lung cancer (2.21 million, third and colon and rectal cancer (1.93 million), fourth prostate with 7.3% (1.41 million), non-melanoma skin with 6.2% (1.2 million), and stomach (1.09 million), new cases in the world (WORLD HEALTH ORGANIZATION, 2023).



Data source: GLOBOCAN 2020
Map production: IARC
(http://gco.iarc.fr/today)
World Health Organization

https://gco.iarc.fr/today/online-analysis-map

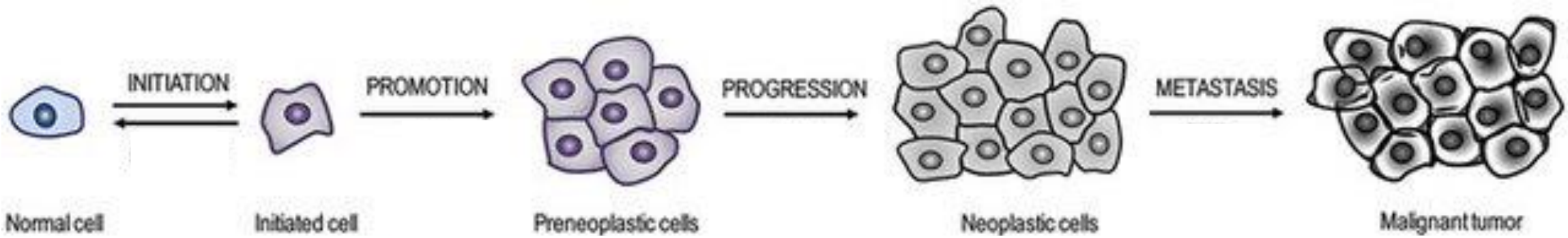
Cancer

In Brazil, the estimate for the 2023-2025 triennium indicates 704 thousand new cases of cancer, that is, 483 thousand excluding cases of non-melanoma skin cancer. For these numbers, an estimated 10.5% of female breast cancers (74 thousand), prostate with 10.2% (72 thousand), colon and rectum with 6.5% (46 thousand), lung cancer with 4.6% (32 thousand), with 31.3% (220 thousand), non-melanoma skin cancer, and 3.1% and (21 thousand), for stomach cancer (INSTITUTO NATIONAL DE CANCER – INCA Estimate 2023, 2023).

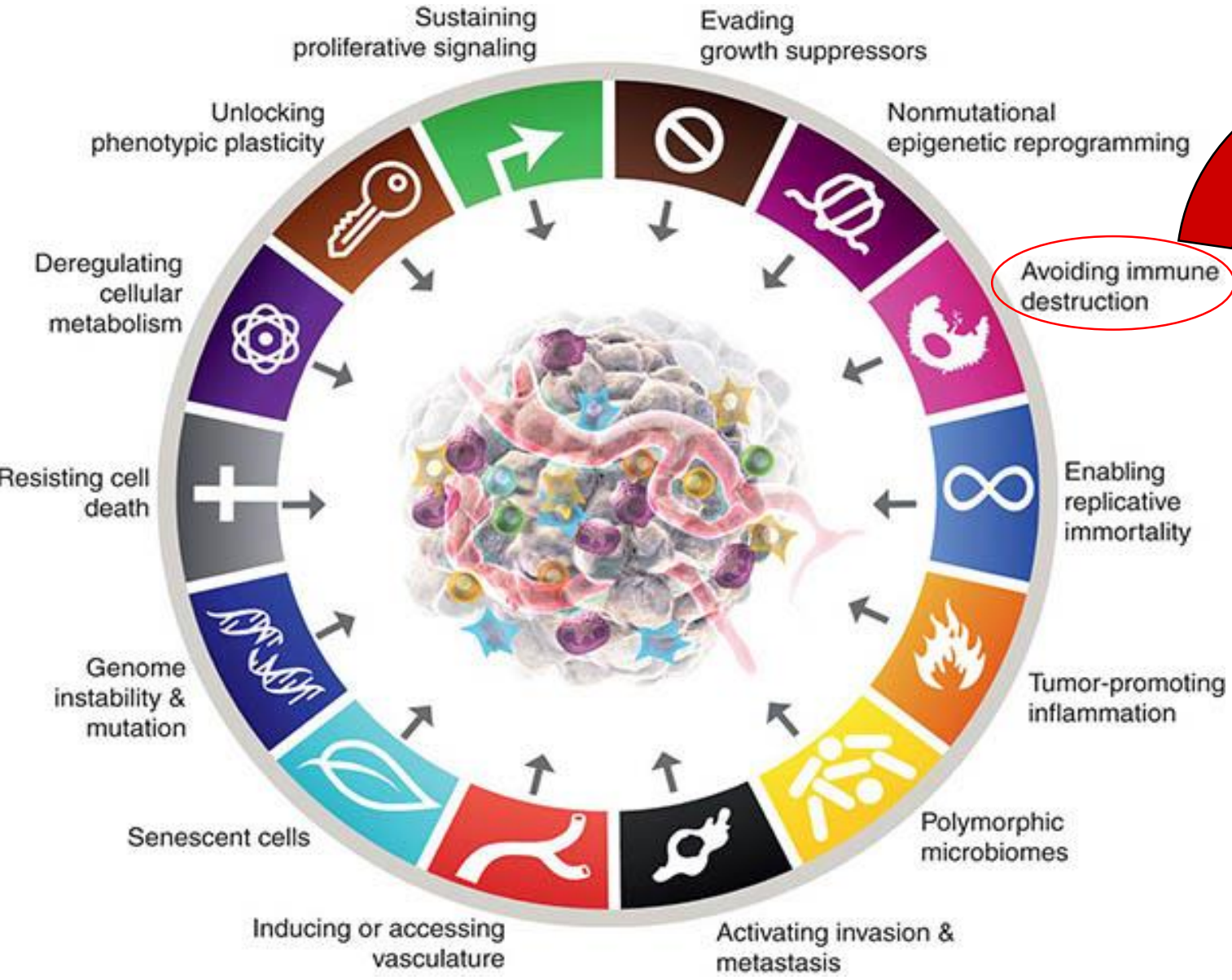
Brazil
brasilescola.uol.com.br

INCA
INSTITUTO NACIONAL DE CÂNCER

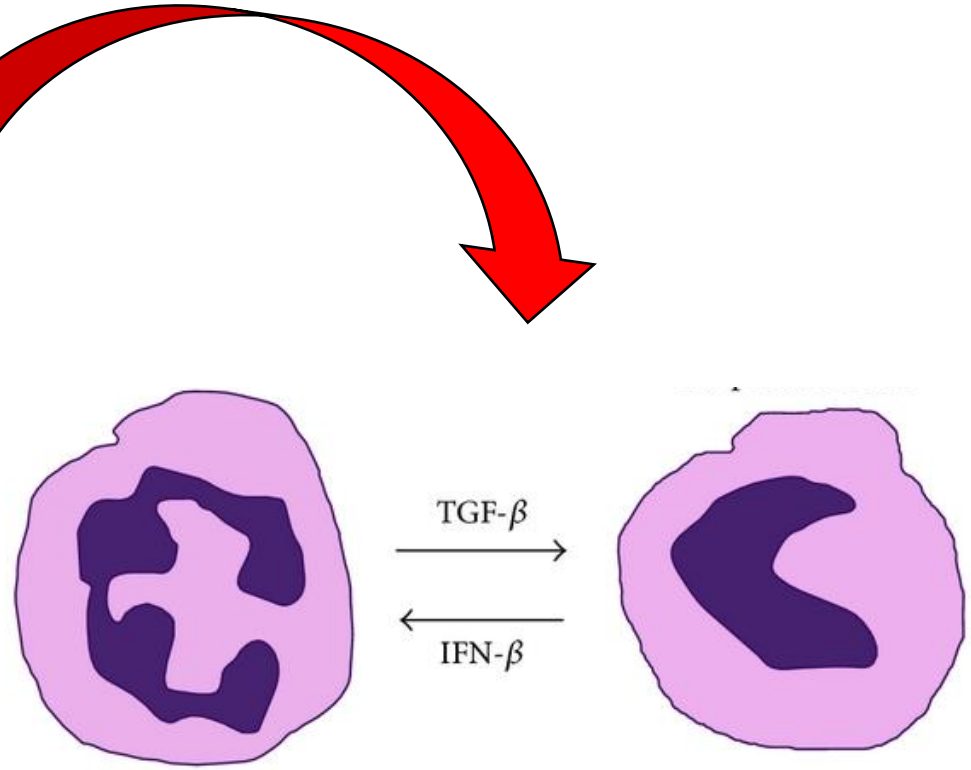
In cancer, there is a loss of control over the mechanisms of proliferation, differentiation and cell death (Moffat et al., 2000; Hanahan; Weinberg, 2011).



Siddiqui, I.A., Sanna, V. et al (2015)



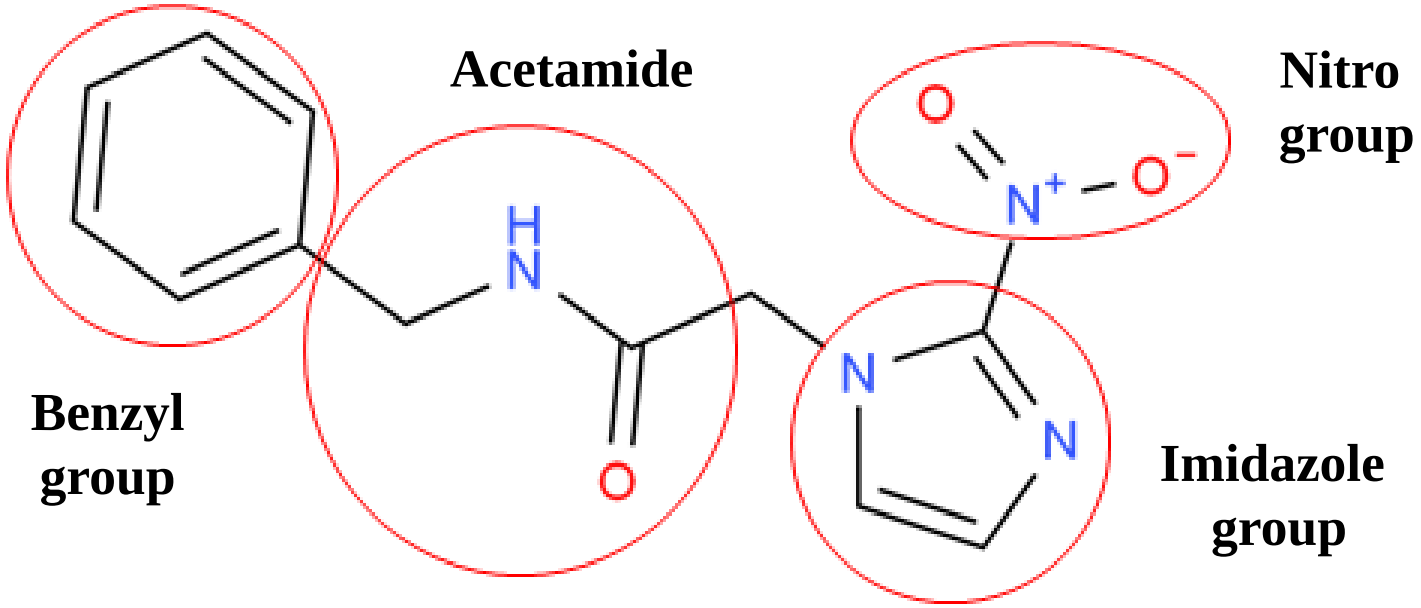
https://www.aacr.org/blog/2022/01/21/new-dimensions-in-cancer-biology-updated-hallmarks-of-cancer-published/



Granot, Z.. Jablonska, J. (2015),

BENZNIDAZOLE - BZN

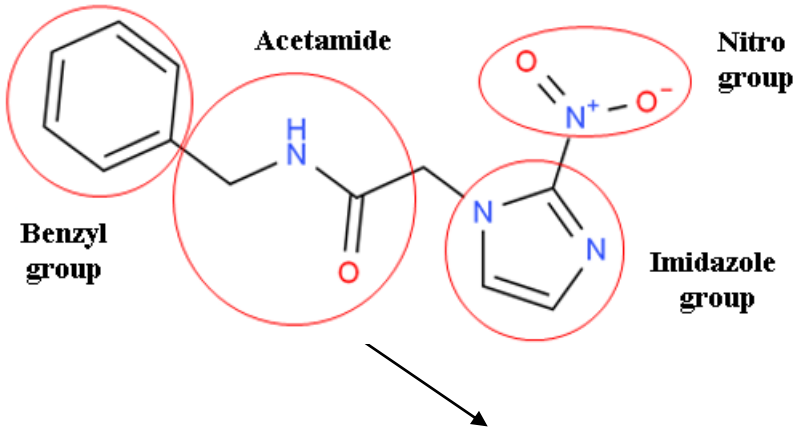
N-benzyl-2-(2-nitroimidazol-1-yl) acetamide



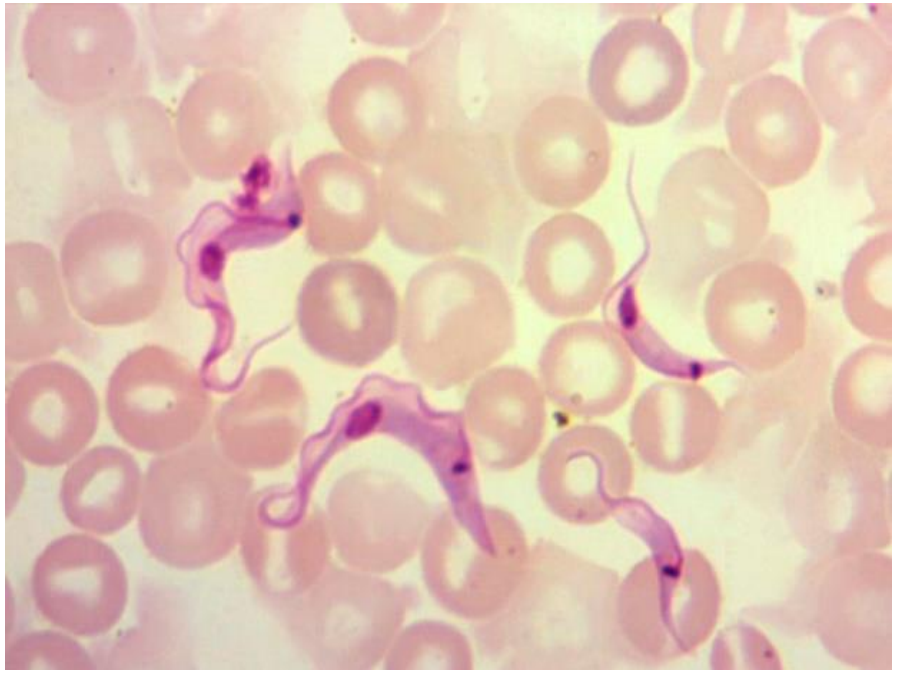
Reactive Metabolite

Prodrug – activation by CYP 450

BZN

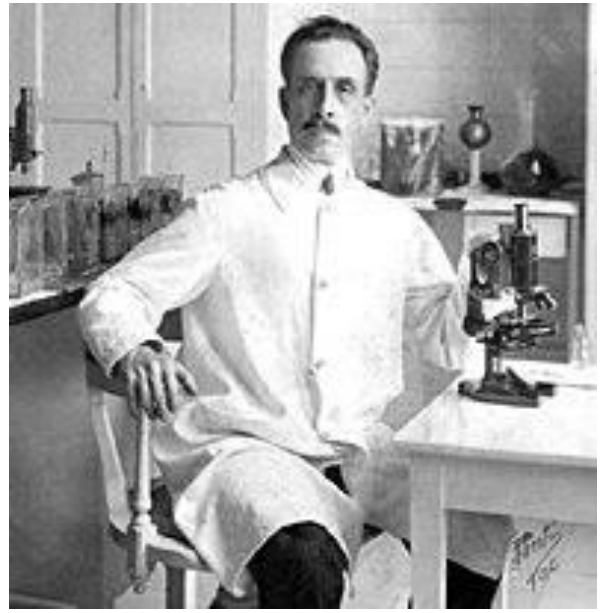


American trypanosomiasis, called Chagas disease in honor of its discoverer, Brazilian researcher Carlos Chagas, is an important parasitic disease resulting from infection by the hemoflagellate protozoan parasite Trypanosoma cruzi, with triatomine insects as vectors



Trypanosoma cruzi
https://www.icc.fiocruz.br/doenca-de-chagas/

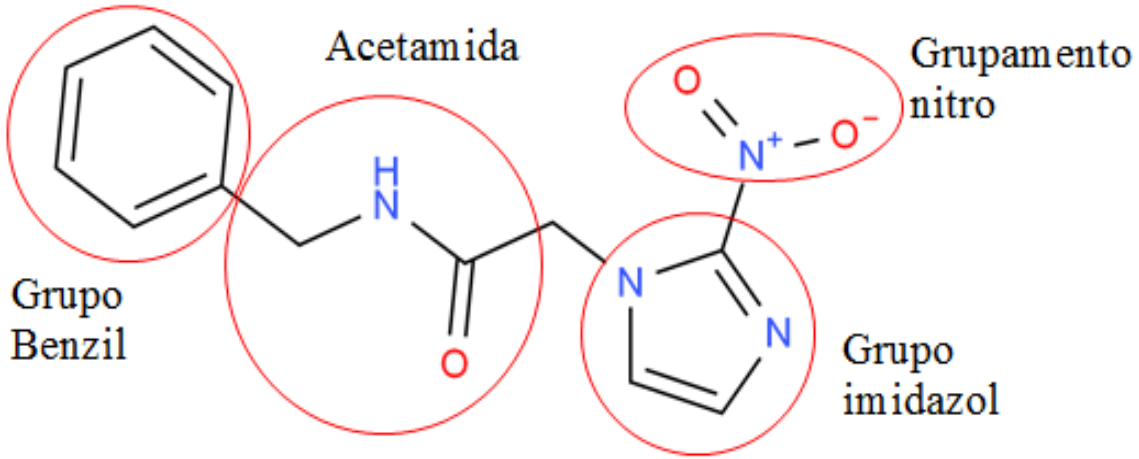
Panstrongylus megistus
https://revistapesquisa.fapesp.br/focos-de-barbeiro-na-cidade-de-sao-paulo/



Carlos Chagas 1878 -1934

BENZNIDAZOLE -BZN

N-benzyl-2-(2-nitroimidazol-1-yl) acetamide



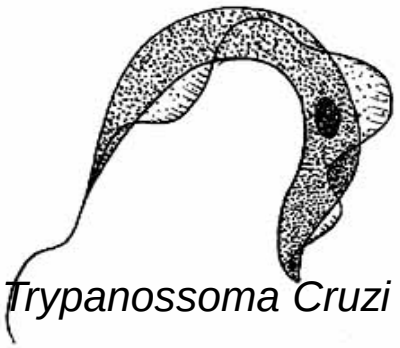
→ BZN came into medical in 1971 year.

→ Knowledge acquired through 50 years of use

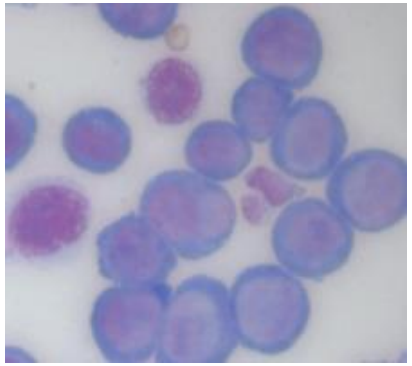
Reactive Metabolite
Prodrug – activation by CYP 450

“The free racidals produced act on DNA, proteins and lipids leading to importante Oxidative damage to biological macromolecules” (URBINA; DO CAMPO, 2003)”

Therefore...



Trypanossoma Cruzi



Células Tumor Ascítico de Ehrlich

Tumor cells and parasites exhibit metabolic similarities

→ Uneven antioxidant defense

→ High proliferation

→ Evasion of the immune response.

(M.Q. KLINKERT, V. HEUSSLER, 2006)

We used PASS software to predict the interaction of BZN (PubChem Cid: 31593), with molecular target groups

Molecular Target Group

TARGET	NUMBER OS TARGETS ASSOCIATED WITH GO BIOLOGICAL PROCESSES
RESPONSE TO STRESS	40
SIGNAL TRANSDUCTION	55
METABOLIC PROCESS	35
IMMUNE SYSTEM PROCESS	27

We used PASS software to predict the interaction of BZN (PubChem Cid: 31593), with possible biological activities

Predict possible biological activities

P_a	P_i	Activity
0.888	0.001	Chemosensitizer
0.827	0.002	Radiosensitizer
0.633	0.003	Oxidizing agent
0.409	0.008	Antineoplastic enhancer
0.409	0.015	Antioplastic (Pancreatic cancer)
0.482	0.093	Calcium channel (voltage-sensitive)activator
0.404	0.036	Antineoplastic (solid Tumors)

These results corroborate with Zeferino et al., 2019, which demonstrate the ability of BZN to inhibit tumor growth, generating reactive oxygen species, increasing calcium influx, activating apoptosis

Investigational New Drugs (2020) 38:785–799
https://doi.org/10.1007/s10637-019-00820-5

PHASE I STUDIES

Check for updates

Targeting ROS overgeneration by N-benzyl-2-nitro-1-imidazole-acetamide as a potential therapeutic reposition approach for cancer therapy

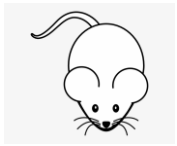
Rodrigo C. Zeferino[1] · Nádia S. R. S. Mota[1] · Valdelúcia M. A. S. Grinevicius[1] · Karina B. Filipe[2] · Paola M. Sulis[1] · Fátima R. M. B. Silva[1] · Danilo W. Filho[3] · Claus T. Pich[4] · Rozangela C. Pedrosa[1]

Design of experiments *in vivo*

Experimental Groups

Control (1%DMSO) | **MTX (2,5mg/kg)** | **BZN (10mg/kg)**

Protocol CEUA PP00784



Day 0 → **Day 1** → **Treatment** (9 days, 50 µL, ip) → **Day 10**

EAC inoculation

200 µL, $5X10^6$ cells
(KVIECINSKI et al., 2008)

n=6

Rapid panoptic and measurement of myeloperoxidase (MPO)activity

It's important to remember that.....

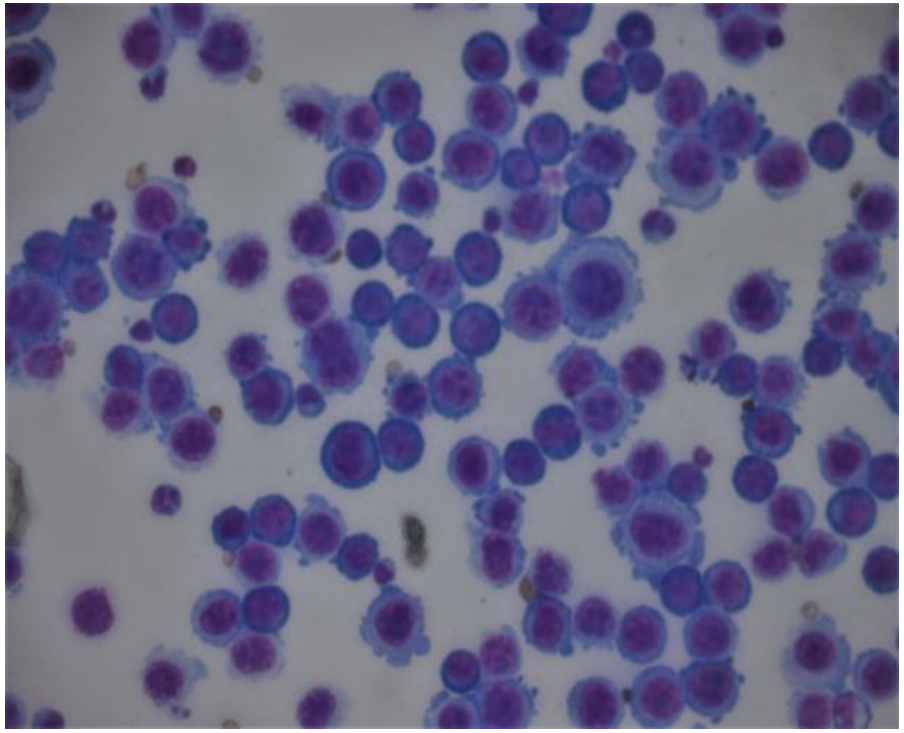
EAC has low expression of histocompatibility complex proteins, demonstrating that cellular immunity is not the main mechanism of host reaction against this tumor (CHEN, L. WATKINS, J.F. 1970).

Cells (%)	EAC cells	Neutrophils		Lymphocyte	Monocyte
		Segmented	Band		
Control	97.50±1.00	1.25±0.50	0.25±0.30	0.33±0.57	1.00±0.57
MTX	97.30±0.56	ND	ND	ND	ND
BZN	92.50±0.57	5.35±1.25	1.75±0.95	0.33±0.47	1.00±0.50

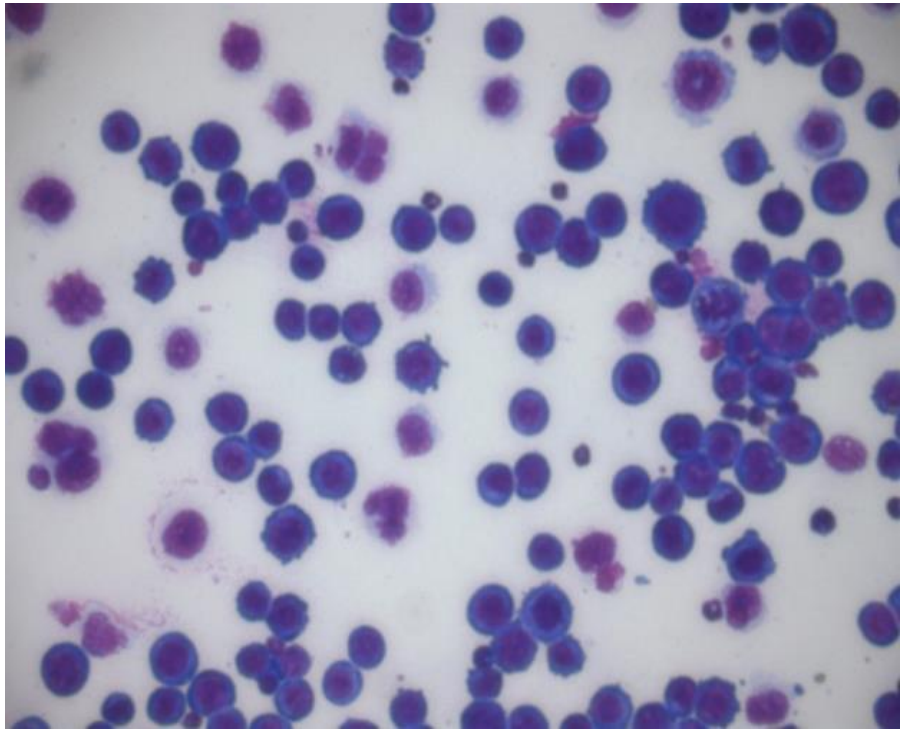
Note: Neutrophils = (Segmented + band)

Increased infiltration of immune system cells in treated animals

Control



BZN



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5-fold **7-fold**

Increased infiltration of immune system cells in treated animals

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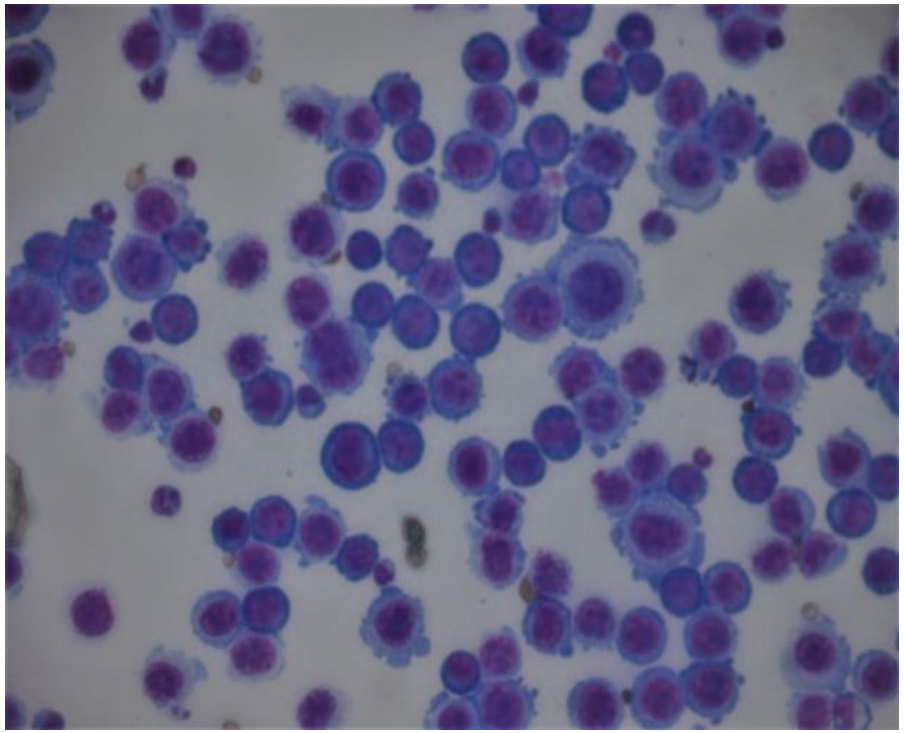
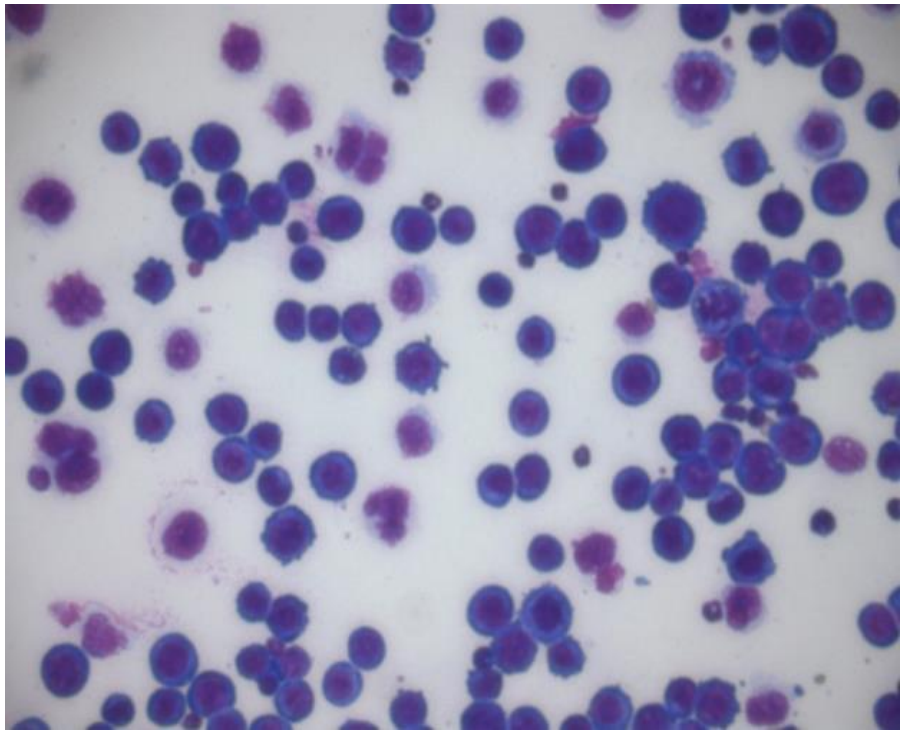


BZN



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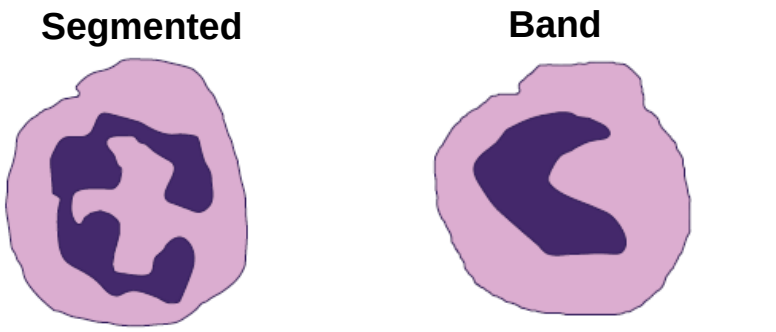
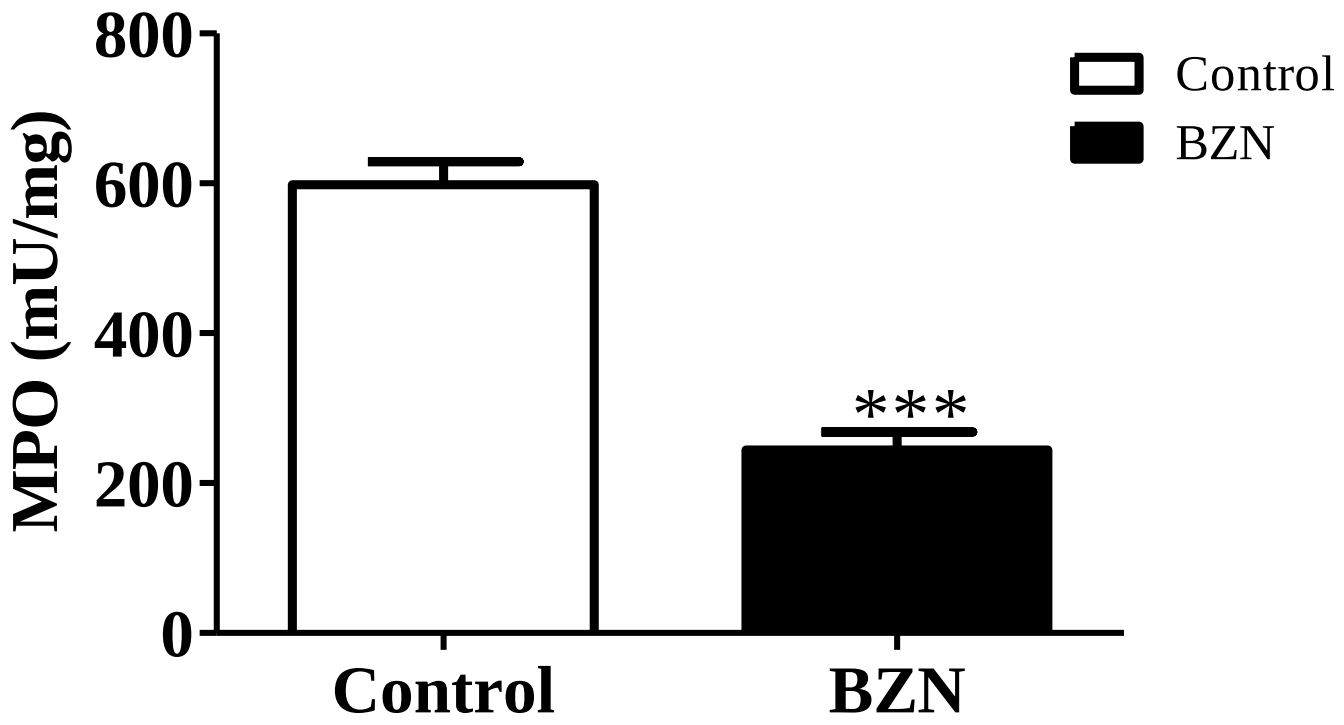
Increased infiltration of immune system cells in treated animals



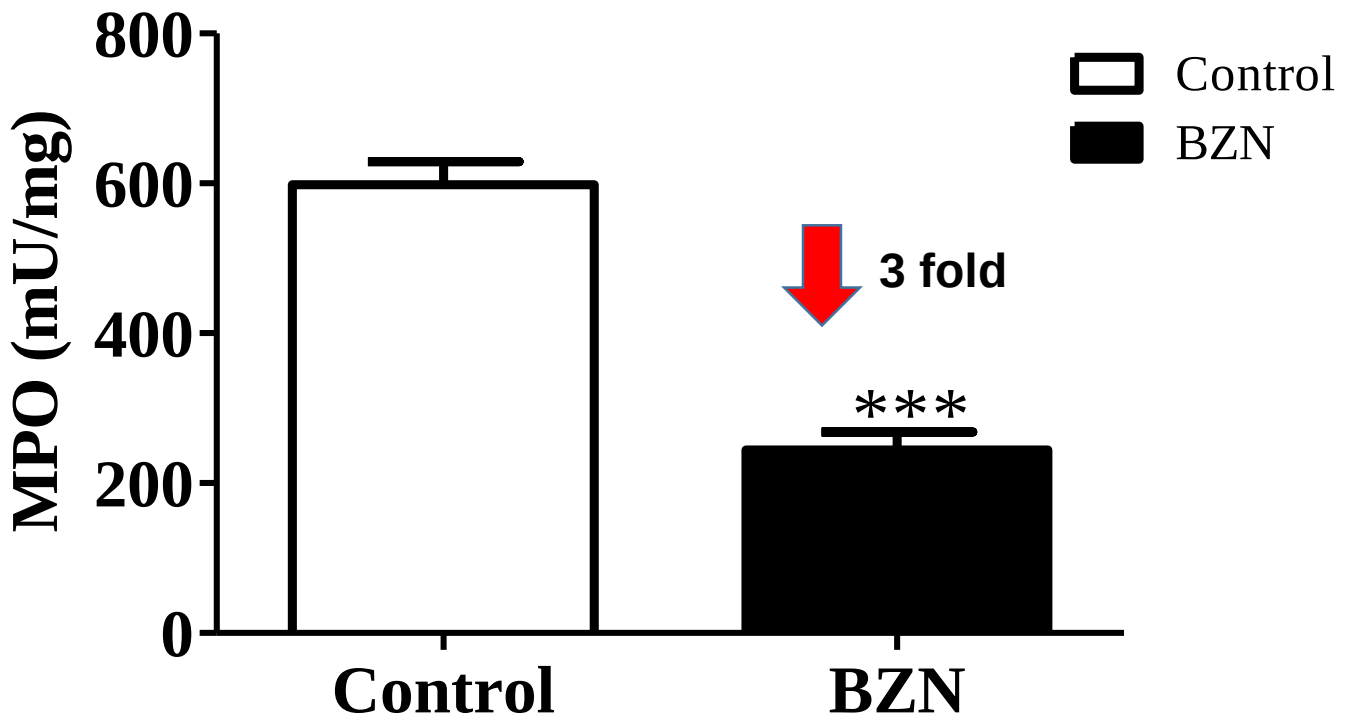
Granot, Z.. Jablonska, J. (2015)

Myeloperoxidase (MPO)



Quantification of immune cells present in ascites fluid collect from EAC bearing BALB-c mice male, n=6; 20±2g weight: The activity of myeloperoxidase (MPO) was attenuated after treatment with BZN 10mg/kg/day. (***) denotes statistical difference when compared to controls ($p<0.001$). Values are expressed as means ± S.E.M, n=6.

Myeloperoxidase (MPO)



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Studie published by Silva, E.L. et al. (1990), suggest that BZN can act as a modulator of the immune system, helping to eliminate Trypanosoma cruzi

Modulation of MPO appears to control the cytotoxic and immunoprotective activity mediated by defense cells (neutrophils), making the cytotoxic action more effective against tumor cells (CHANG, C.Y. et al. 2013)

Similar results were obtained by Lambertucci, F. (2017), who demonstrated the ability of BZN to attenuate the inflammatory response in murine sepsis models.

Therefore, the results demonstrate that BZN can act by modulating the immune system (Nuetrophils), facilitating the cytotoxic action of these cells against the tumor

Conclusion

In view of the arguments presented, BZN presented important characteristics to be a possible promising candidate for the repositioning of drugs to combat tumor cells, however, we need more studies on its immunomodulation capacity.

Acknowledgment

PGFar/UFSC
PROGRAMA DE PÓS-GRADUAÇÃO EM FARMÁCIA
15 ANOS

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CAPES

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Thank you very much for your attention

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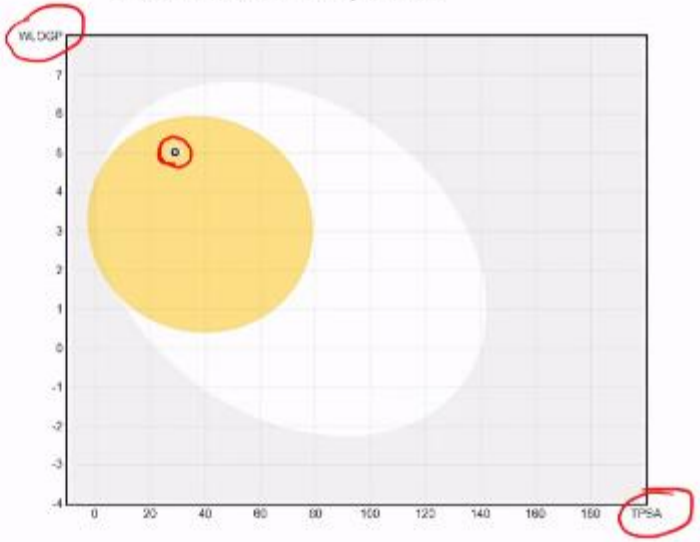
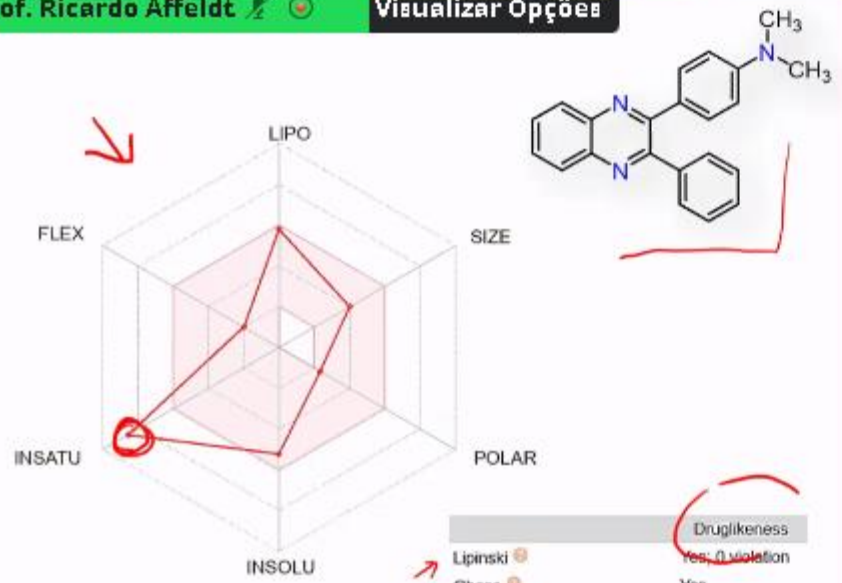
SIB Swiss Institute of Bioinformatics

SwissADME

- Passive permeation through BBB
- Oral bioavailability radar



*Also, compound was not found on SwissSimilarity



	Druglikeness
Lipinski	Yes; 0 violation
Ghose	Yes
Veber	Yes
Egan	Yes
Muegge	Yes
Bioavailability Score	0.55

SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci. Rep.* (2017) 7:42717.