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Synthesis and biological evaluation of inducible Nitric Oxide Synthase inhibitors as anticancer agents

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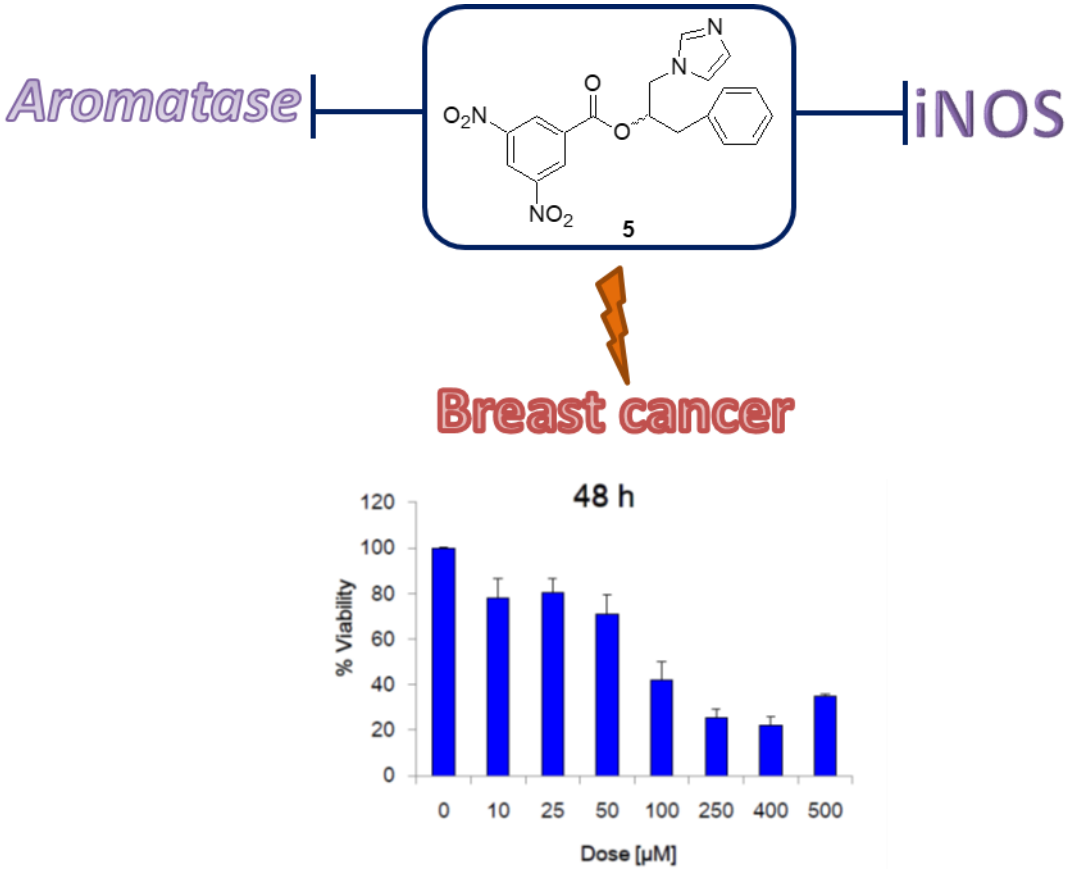
Synthesis and biological evaluation of inducible Nitric Oxide Synthase inhibitors as anticancer agents

Graphical Abstract

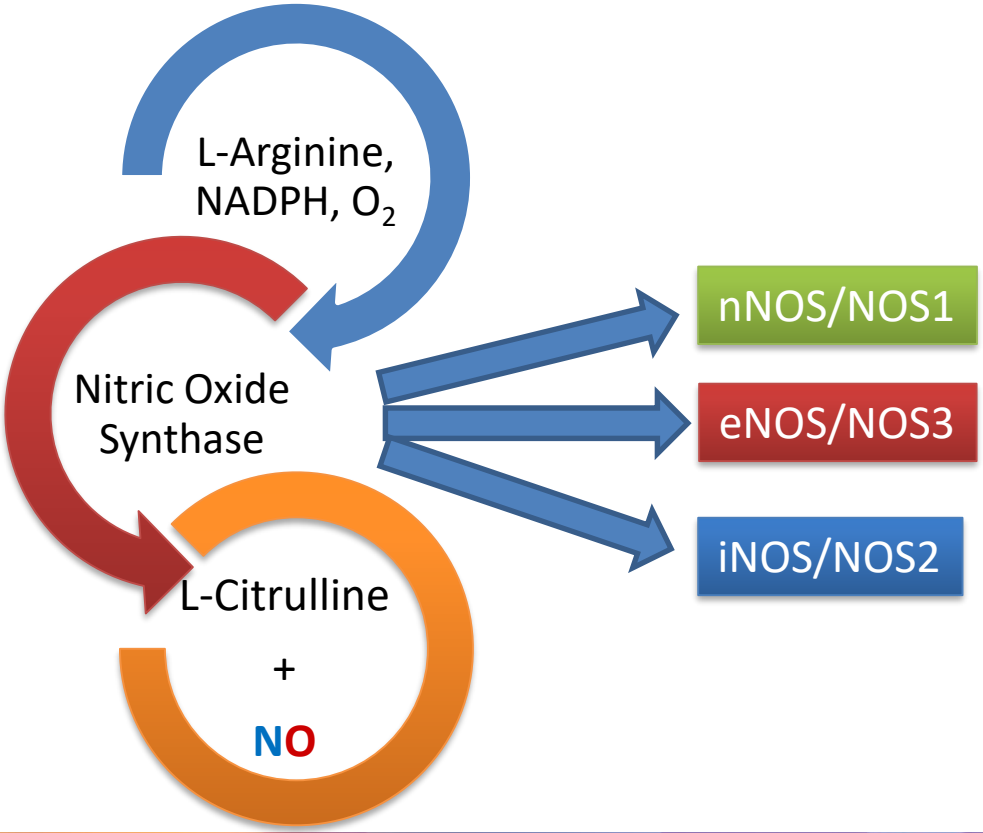


Abstract:
Nitric Oxide (NO) is a free radical signalling molecule, involved in different biological processes and produced by nitric oxide synthases (NOS). There are two constitutive NOS (the endothelial and neuronal ones) and an inducible NOS (iNOS).
In tumour biology, NO has a controversial role, since there is evidence that NO can both inhibit and stimulate tumour cell growth. This response depends on tumour type, genetic background, NO levels and sensibility in the target cells. Correlation between iNOS expression and clinical outcome associated to worse prognosis, was evaluated in different types of tumours. Therefore, inhibition of iNOS has been proposed as a targeted therapy in several cancers, including breast cancer and gliomas.
Our research group is involved in the research of new potent and selective iNOS inhibitors, and we have recently collected evidences of their usefulness as antiglioma agents, compromising in vitro proliferation of cancer cells with selectivity with respect to astrocytes, and ameliorating the effects of the standard therapeutic agent Temozolomide. Moreover, a set of azole-based compounds showed interesting activity both as iNOS and aromatase inhibitors, compromising the MCF-7 breast cell line proliferation, and thereby suggesting their potential application in a polypharmacological approach. In this presentation results obtained from these studies will be shared.

Keywords: Cancer; Inhibition; Nitric Oxide; synthesis

Nitric Oxide

- Endogenous gaseous free radical
- Lipophilic
- Reactive
- Second messanger



iNOS: a valuable target for the cancer therapy

Critical Review

IUBMB *Life*, 64(8): 676–683, August 2012

NOS-2 Signaling and Cancer Therapy

Ka Bian, Farshid Ghassemi, Alex Sotolongo, Alan Siu, Lauren Shauger, Alex Kots and Ferid Murad

Current Molecular Medicine 2013, *13*, 1241-1249

iNOS: A Potential Therapeutic Target for Malignant Glioma

A. Jahani-Asl and A. Bonni*

Inhibition of iNOS as a novel effective targeted therapy against triple-negative breast cancer

Sergio Granados-Principal[1], Yi Liu[1], Maria L Guevara[2], Elvin Blanco[3], Dong Soon Choi[1], Wei Qian[1], Tejal Patel[1], Angel A Rodriguez[1], Joseph Cusimano[4], Heidi L Weiss[5], Hong Zhao[6], Melissa D Landis[1], Bhuvanesh Dave[1], Steven S Gross[7] and Jenny C Chang[1,6*]

Review

The Potential Role of iNOS in Ovarian Cancer Progression and Chemoresistance

Michal Kielbik, Izabela Szulc-Kielbik and Magdalena Klink *

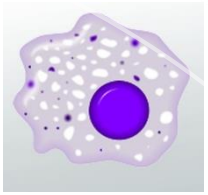
The NOS isoforms: where are the differences?

nNOS (NOS-1)

CNS; PNS

Synaptic plasticity;
atypical neurotransmission;
Blood pressure control; et

iNOS (NOS-2)



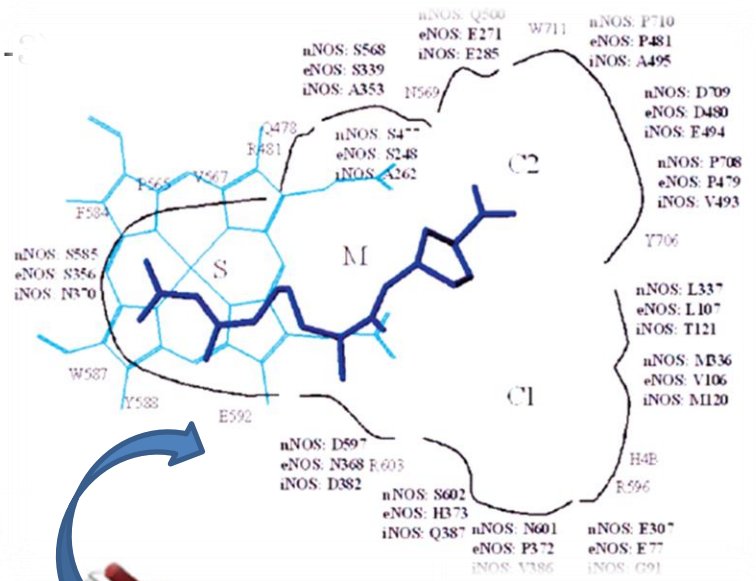
MΦ

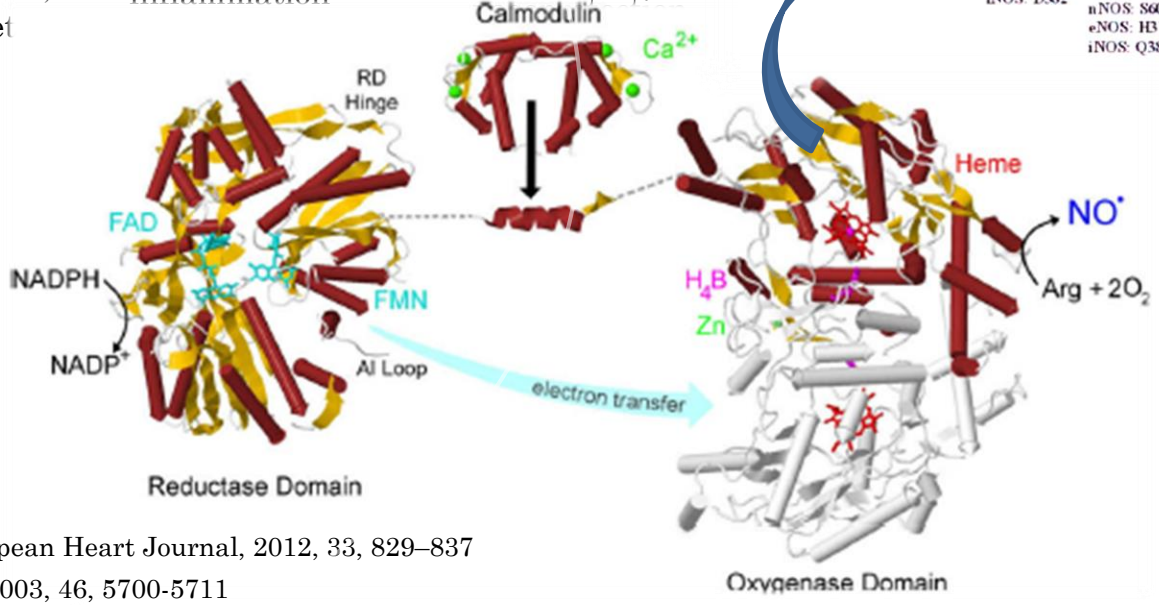
Immune defense;
inflammation

eNOS (NOS-

Endothelium

Vasodilation

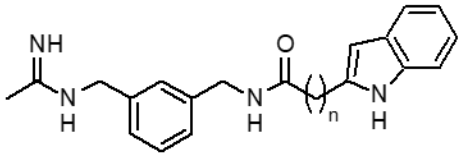




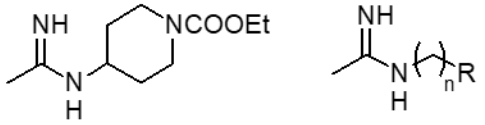
Forstermann, U. et al; European Heart Journal, 2012, 33, 829–837

Ji, H. et al; J. Med. Chem. 2003, 46, 5700-5711

Acetamidines as iNOS inhibitors: a quick view of our background



Fantacuzzi, M. et al. J. Pharm. Biomed. Analysis, 2016, 120, 419–424



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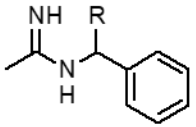

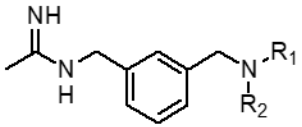

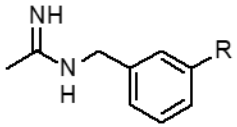

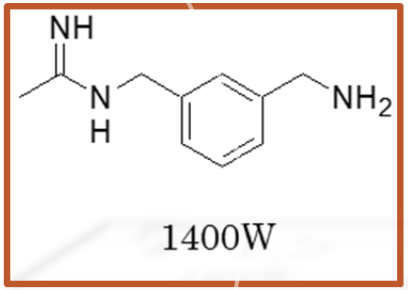

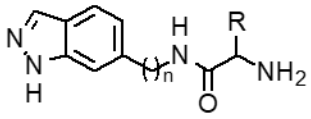

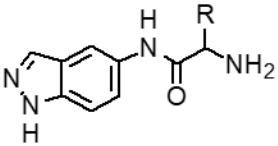

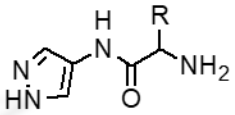

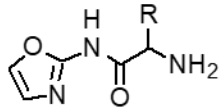

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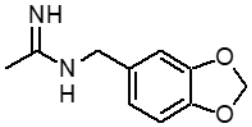

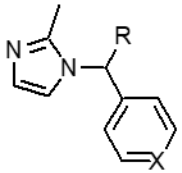

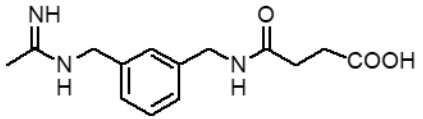

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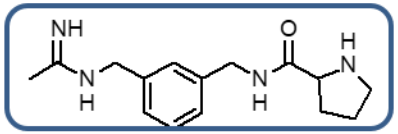

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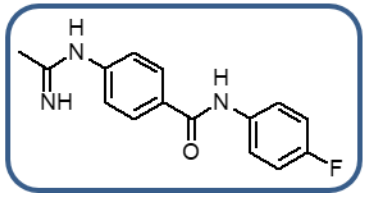
Fantacuzzi, M. et al.ChemMedChem 2011, 6, 1203 – 1206



Maccallini, C. et al. ACS Med. Chem. Lett. 2020, 11, 1470−1475

CM544: a new acetamidine blocking glioma cells

European Journal of Medicinal Chemistry 152 (2018) 53–64

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Discovery of *N*-{3-[(ethanimidoylamino)methyl]benzyl}-L-prolinamide dihydrochloride: A new potent and selective inhibitor of the inducible nitric oxide synthase as a promising agent for the therapy of malignant glioma

Cristina Maccallini [a,*], Mauro Di Matteo [a], Marialucia Gallorini [a], Monica Montagnani [b], Valentina Graziani [a], Alessandra Ammazzalorso [a], Pasquale Amoia [a], Barbara De Filippis [a], Sara Di Silvestre [c], Marialuigia Fantacuzzi [a], Letizia Giampietro [a], Maria A. Potenza [b], Nazzareno Re [a], Assunta Pandolfi [c], Amelia Cataldi [a], Rosa Amoroso [a]

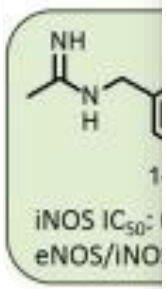
International Journal of *Molecular Sciences*

MDPI

Article

The Selective Acetamidine-Based iNOS Inhibitor CM544 Reduces Glioma Cell Proliferation by Enhancing PARP-1 Cleavage In Vitro

Marialucia Gallorini, Cristina Maccallini *, Alessandra Ammazzalorso, Pasquale Amoia, Barbara De Filippis, Marialuigia Fantacuzzi, Letizia Giampietro, Amelia Cataldi and Rosa Amoroso



Developing new iNOS inhibitors blocking glioma cells

ACS Medicinal Chemistry Letters

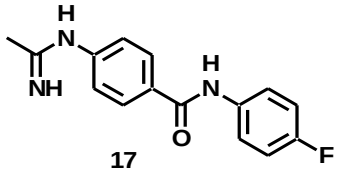
ACS Med. Chem. Lett. 2020, 11, 1470−1475

pubs.acs.org/acsmedchemlett

Letter

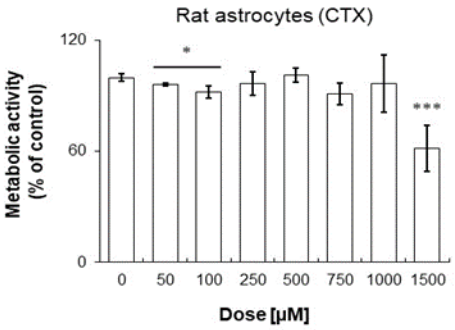
Antiglioma Activity of Aryl and Amido-Aryl Acetamidine Derivatives Targeting iNOS: Synthesis and Biological Evaluation

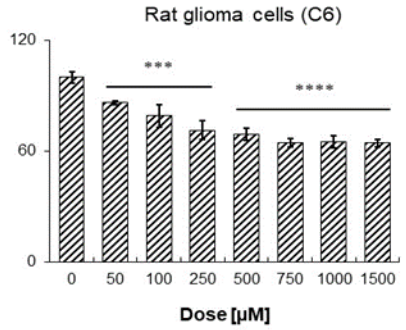
Cristina Maccallini,* Fabio Arias, Marialucia Gallorini, Pasquale Amoia, Alessandra Ammazzalorso, Barbara De Filippis, Marialuigia Fantacuzzi, Letizia Giampietro, Amelia Cataldi, María Encarnación Camacho, and Rosa Amoroso

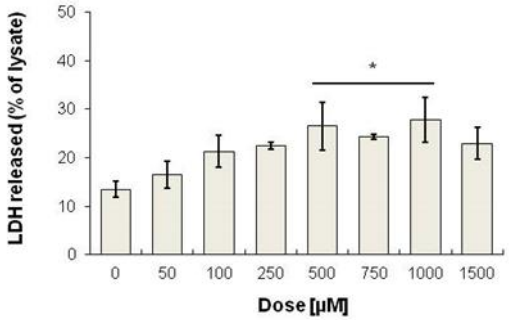


17

Cpd	IC_{50}(μM)[a]			Selectivity ratio	
	hiNOS	hnNOS	heNOS	nNOS/iNOS	eNOS/iNOS
17	0.011±0.02	6.2±0.08	>10	563	>900
1400W	0.080±0.002	7.8±0.04	304±2	72	3800





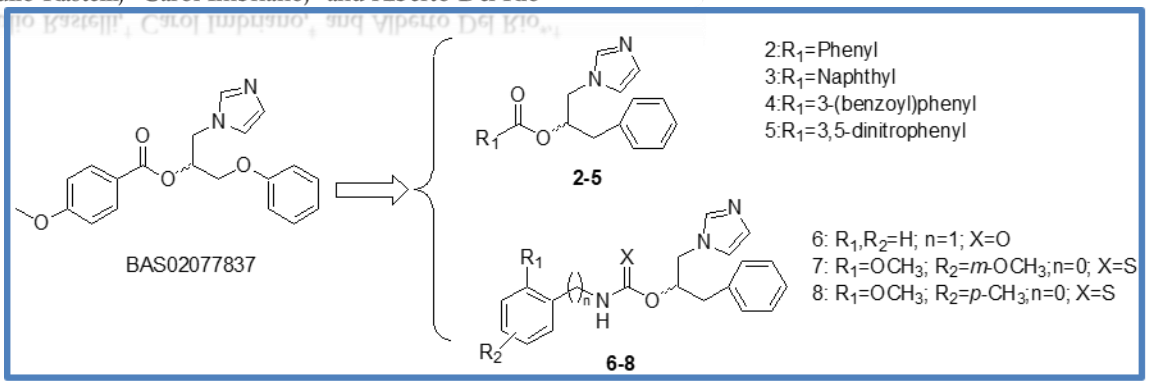


Aim of the work: *developing new iNOS and aromatase inhibitors as dual agents targeting breast cancer*

dx.doi.org/10.1021/jm2000689 | *J. Med. Chem.* 2011, 54, 4006–4017

Structure-Based Design of Potent Aromatase Inhibitors by High-Throughput Docking

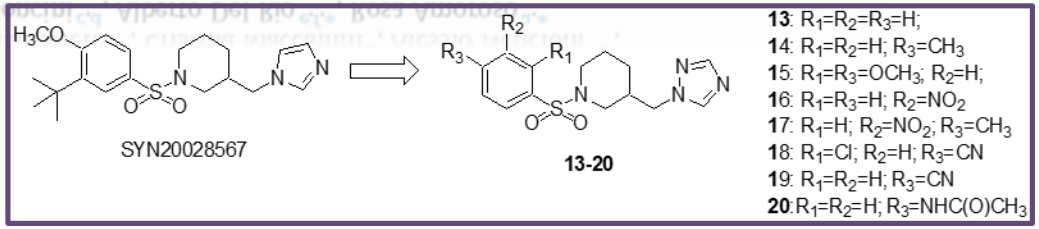
Fabiana Caporuscio,[†] Giulio Rastelli,[†] Carol Imbriano,[‡] and Alberto Del Rio*[,†]



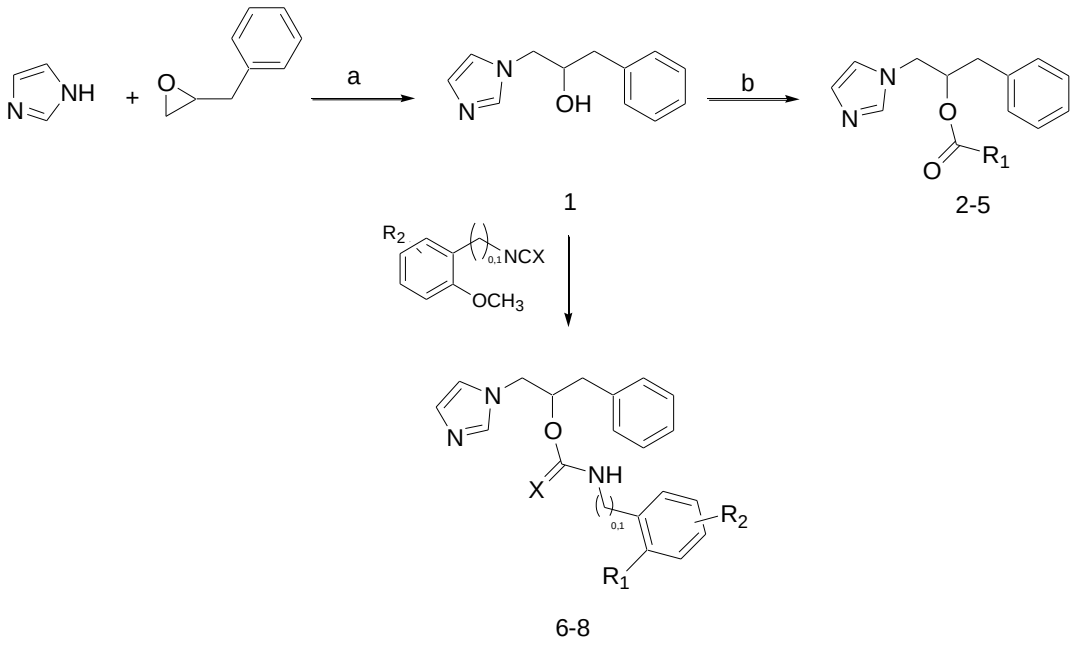
Bioorganic & Medicinal Chemistry Letters 26 (2016) 3192–3194

Synthesis and biological characterization of 3-(imidazol-1-ylmethyl) piperidine sulfonamides as aromatase inhibitors

Mauro Di Matteo [a], Alessandra Ammazzalorso [a], Federico Andreoli [b], Irene Caffa [c,d], Barbara De Filippis [a], Marialuigia Fantacuzzi [a], Letizia Giampietro [a], Cristina Maccallini [a], Alessio Nencioni [c,d], Marco Daniele Parenti [e], Debora Soncini [c,d], Alberto Del Rio [e,f,*], Rosa Amoroso [a,*]

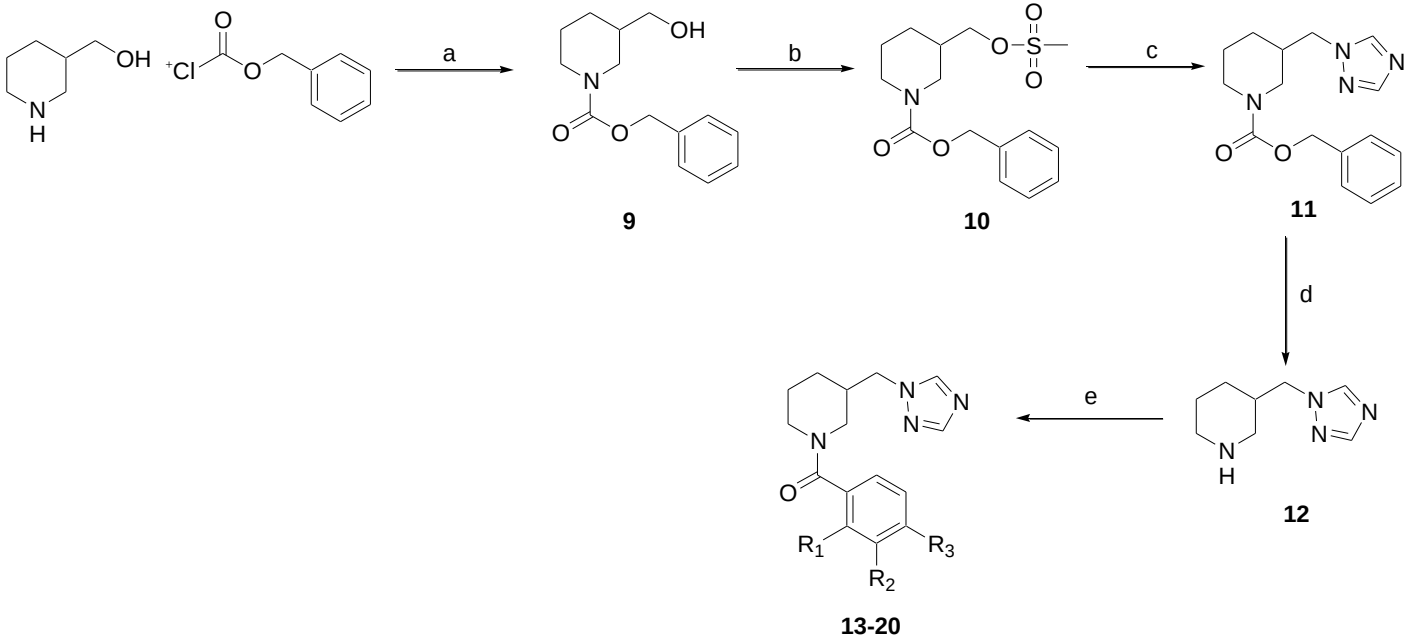


Synthesis of 2-5 and 6-8



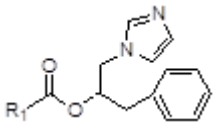
Scheme 1. Reagents and conditions: a. 60°C, 12h; b. aryl-carboxylic acid, DMAP, EDC, CH_3CN dry, N_2, from 0°C to r.t., 18-22h; c. Et_3N, CH_3CN_{dry}, N_2, r.t., 18h or NaH 60%, DMF_{dry}, N_2, from 0°C to r.t., 20h

Synthesis of 13-20



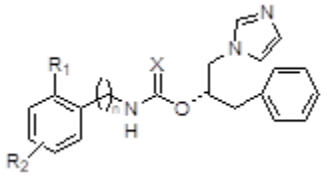
Scheme 2. Reagents and conditions. a) NEt_3, DCM, from 0°C to r.t., 24h. b) Mesylchloride, NEt_3, DCM, r.t., 1h. c) Triazole, NaH 60% mineral oil, DMF dry, N_2, 100°C, 8h. d) H_2, Pd/C, CH_3OH dry, N_2, r.t., 6h. e) $ArSO_2Cl$, NEt_3, DCM dry, N_2, 2h at 0°C and 2h at r.t.

Results: Biological evaluation of 2-5 and 6-8



Cpd	R_1	Aromatase %Inhibition*	iNOS % Inhibition*
2	Phenyl	77	100
3	Naphtyl	13	0
4	3-(Benzoyl)phenyl	36	47
5	3,5-dinitro-phenyl	82	100

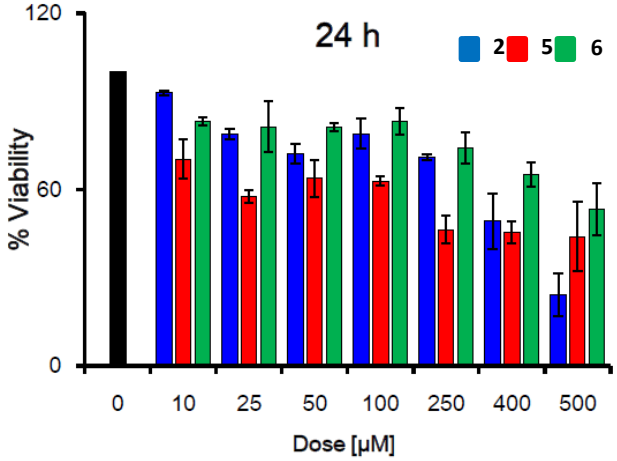
*@ 1μM compound.Data are mean of duplicate experiments. S.D. was within 10%

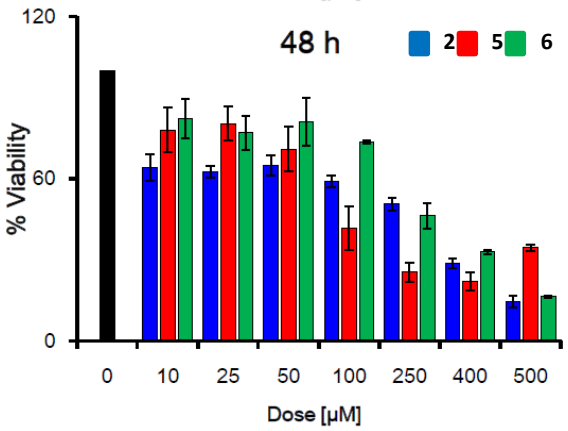


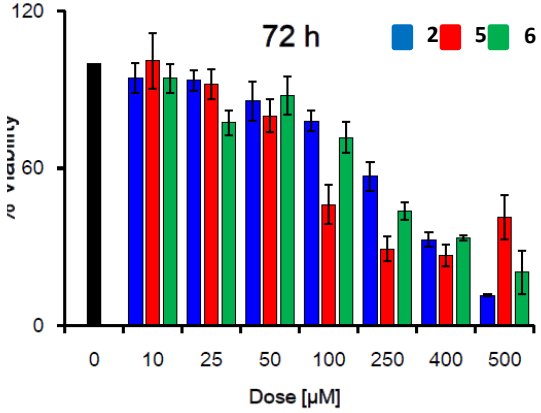
Cpd	R_1	R_2	n	X	Aromatase %Inhibition*	iNOS % Inhibition*
6	H	H	1	O	72	52
7	OCH_3	OCH_3	0	S	15	7
8	OCH_3	CH_3	0	S	54	12

*@ 1μM compound.Data are mean of duplicate experiments. S.D. was within 10%

MCF-7 viability assay

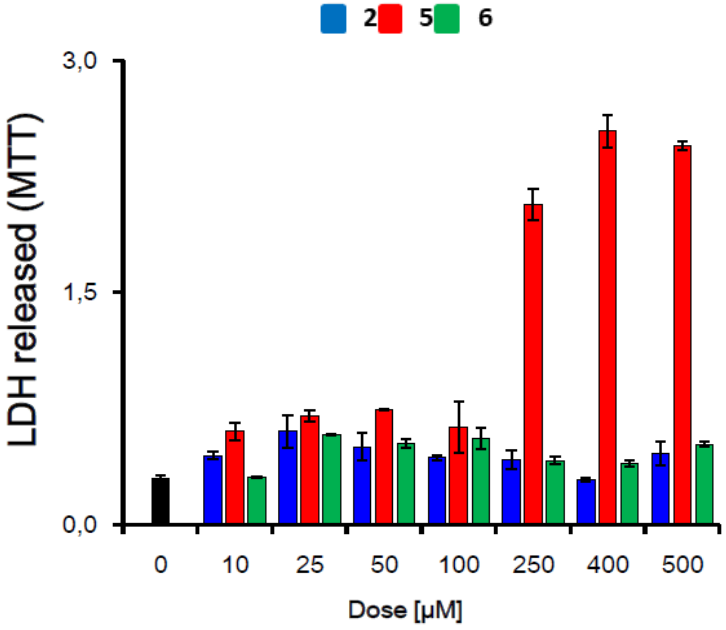




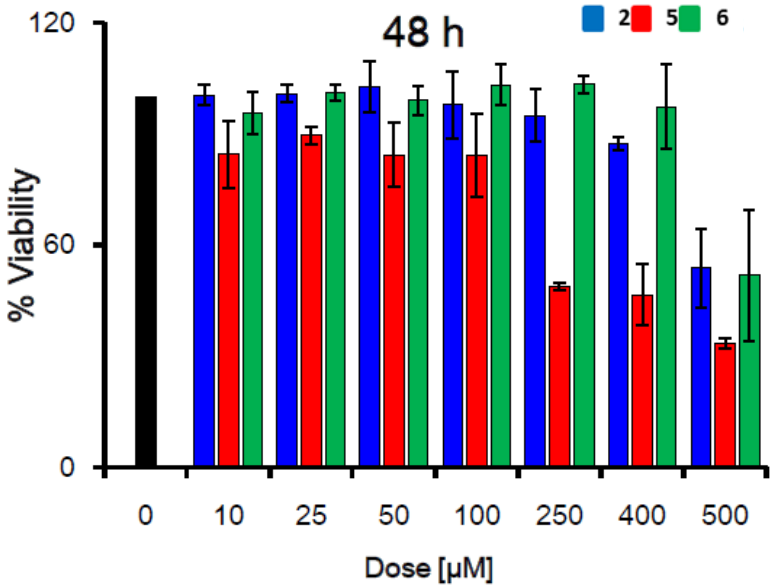


Results: Biological evaluation of 2-5 and 6

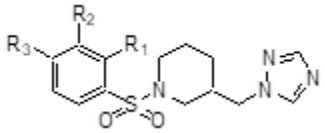
Cytotoxicity (MFC-7)



Selectivity *vs* human gingival fibroblasts



Results: Biological evaluation of 13-20



Cpd	R_1	R_2	R_3	Aromatase %Inhibition*	iNOS % Inhibition*
13	H	H	H	29	27
14	H	H	CH_3	32	22
15	OCH_3	H	OCH_3	62	18
16	H	NO_2	H	63	47
17	H	NO_2	CH_3	25	39
18	Cl	H	CN	55	14
19	H	H	CN	48	16
20	H	H	$NHC(O)CH_3$	61	0

*Data are mean of duplicate experiments. S.D. was within 10%

Conclusions

- ✓ iNOS is a potential biological target for the treatment of cancer.
- ✓ Different iNOS inhibitors were disclosed showing antiproliferative effects on rat glioma cells, without affecting control astrocytes.
- ✓ New iNOS inhibitors were synthesized with dual activity also against aromatase.
- ✓ Among the most potent compounds of the series, molecules **2**, **5**, and **6** demonstrated antiproliferative and cytotoxic effects on MCF-7 breast cancer cell line, although cell selectivity was preliminarly observed only for molecules **2** and **6**.
- ✓ Effects on further human breast cancer cell lines will be evaluated in the next future to confirm the therapeutic potential of these azole-based dual agents.

Acknowledgments

Medicinal Chemistry Group
Prof. Rosa Amoroso
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Dr. Barbara De Filippis
Dr. Marialuigia Fantacuzzi
Dr. Letizia Giampietro

Biology Group
Prof. Amelia Cataldi
Dr. Marialucia Gallorini

Universidad de Granada

Medicinal Chemistry group
Prof. M. Encarnacion Camacho
Dr. Fabio Arias Bordajandi