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Methoxyphenylimidazolines as potential activators of p53

Chaired by DR. ALFREDO BERZAL-HERRANZ;
Co-Chaired by PROF. DR. MARIA EMÍLIA SOUSA

pharmaceuticals
MDPI

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Lomonosov Moscow State University

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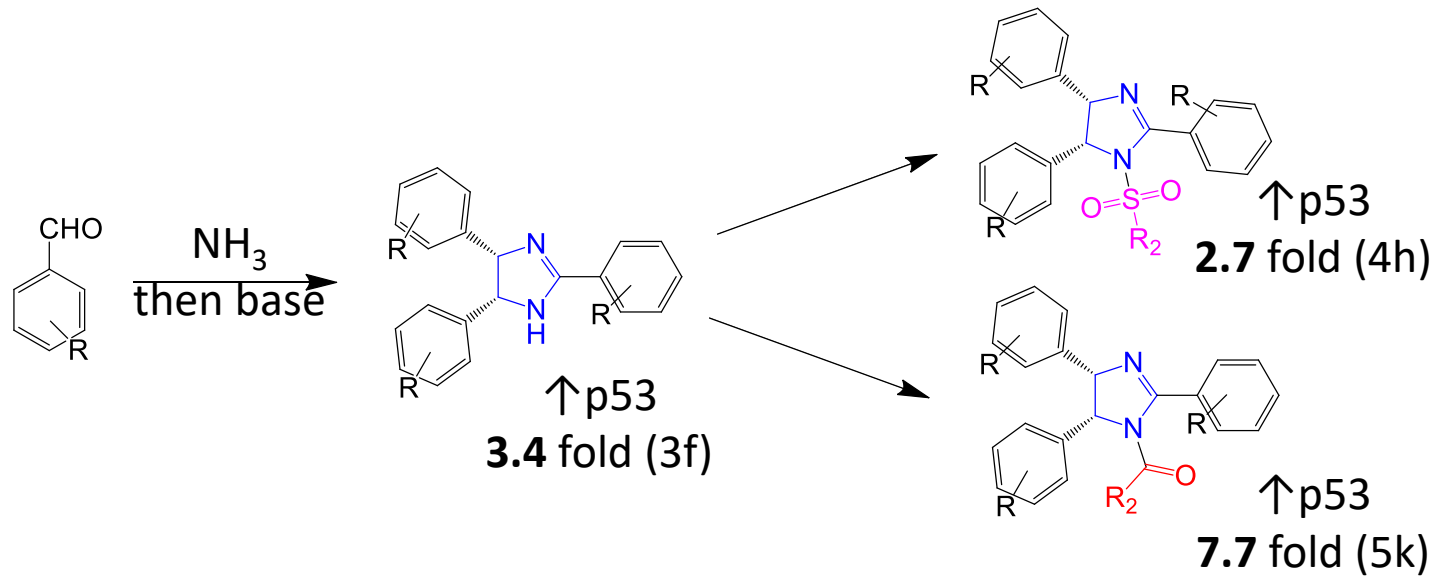
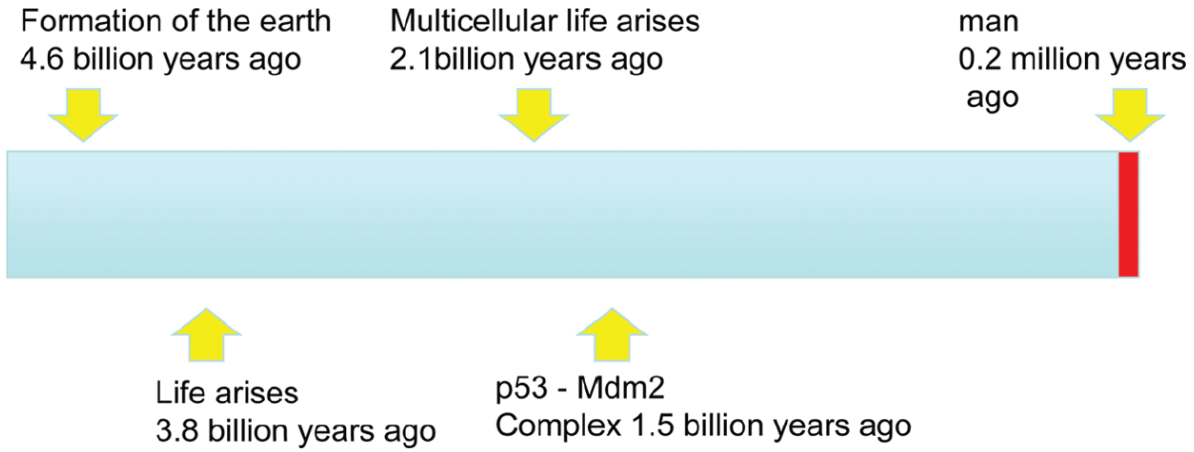
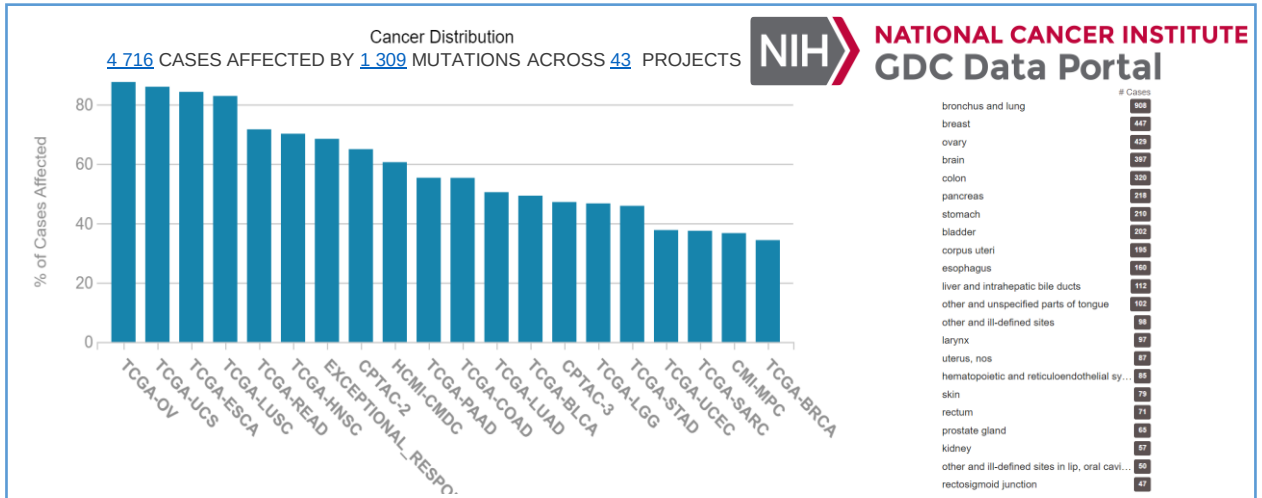
Graphical Abstract



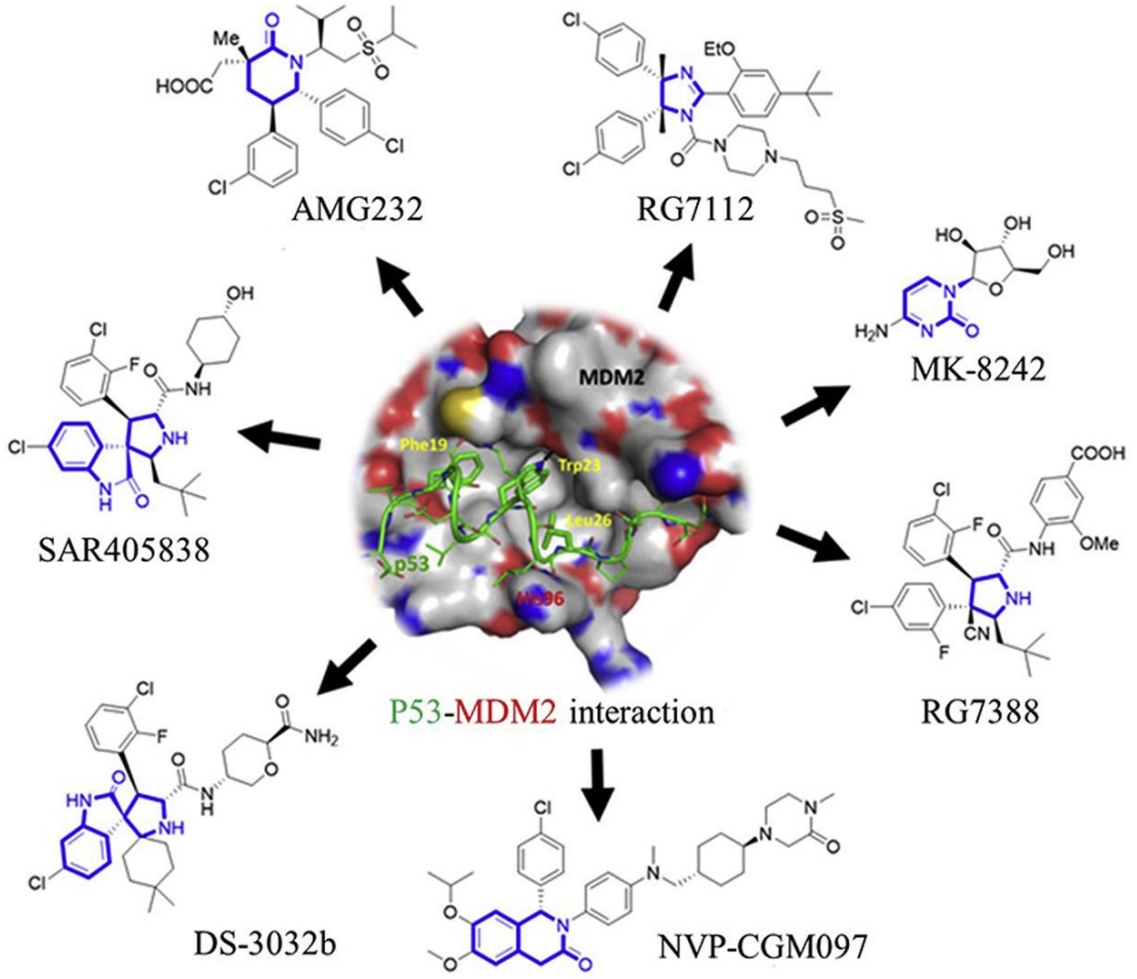
№2. p53/mdm2 interaction



The p53-mdm2 complex on the time scale.
Lane, D. P., & Verma, C. (2012). ***Mdm2 in Evolution***. *Genes & Cancer, 3(3-4), 320–324.* doi:10.1177/1947601912458285

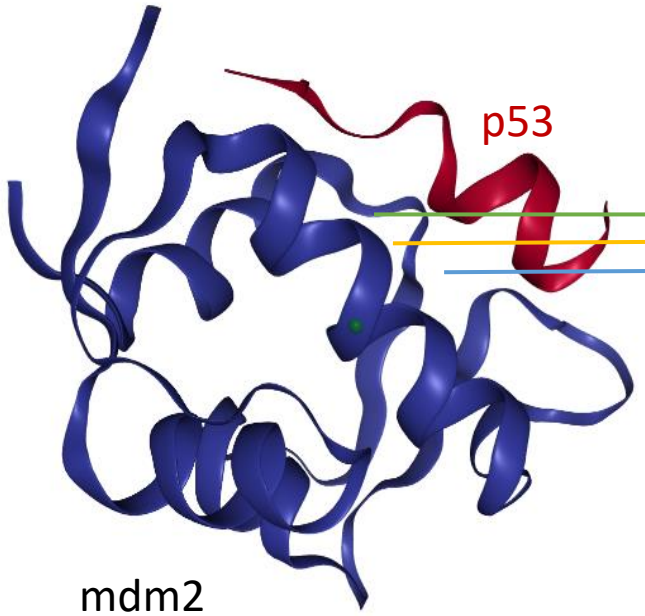


The frequency of mutations of the p53 gene in oncology (y axis), projects of the US National Cancer Institute (x axis). (https://portal.gdc.cancer.gov/)

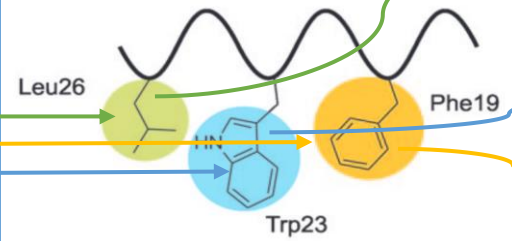


Examples of the main inhibitors of p53-MDM2 interaction.
Yuan Fang, Guochao Liao, Bin Yu, ***Small-molecule MDM2/X inhibitors and PROTAC degraders for cancer therapy: advances and perspectives****, Acta Pharmaceutica Sinica B, Volume 10, Issue 7, 2020, Pages 1253-1278, ISSN 2211-3835, https://doi.org/10.1016/j.apsb.2020.01.003.*

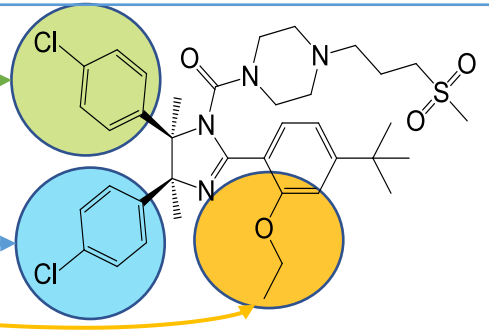
№3. p53/mdm2 inhibitors



PDB:1YCR, Kussie, P. H., Gorina, S., Marechal, V., Elenbaas, B., Moreau, J., Levine, A. J., & Pavletich, N. P. (1996). ***Structure of the MDM2 Oncoprotein Bound to the p53 Tumor Suppressor Transactivation Domain***. *Science, 274(5289), 948–953.* doi:10.1126/science.274.5289.948

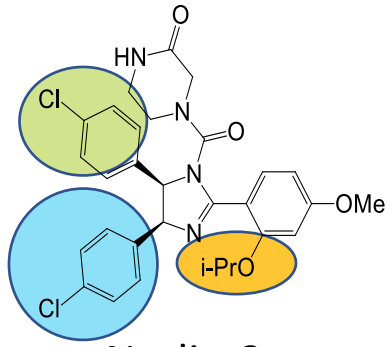


Hydrophobic amino acid residues of the p53 protein in the mdm2/x binding site.



RG7112

Vu, B., Wovkulich, P., Pizzolato, G., Lovey, A., Ding, Q., Jiang, N., … Graves, B. (2013). ***Discovery of RG7112: A Small-Molecule MDM2 Inhibitor in Clinical Development***. *ACS Medicinal Chemistry Letters, 4(5), 466–469.* doi:10.1021/ml4000657

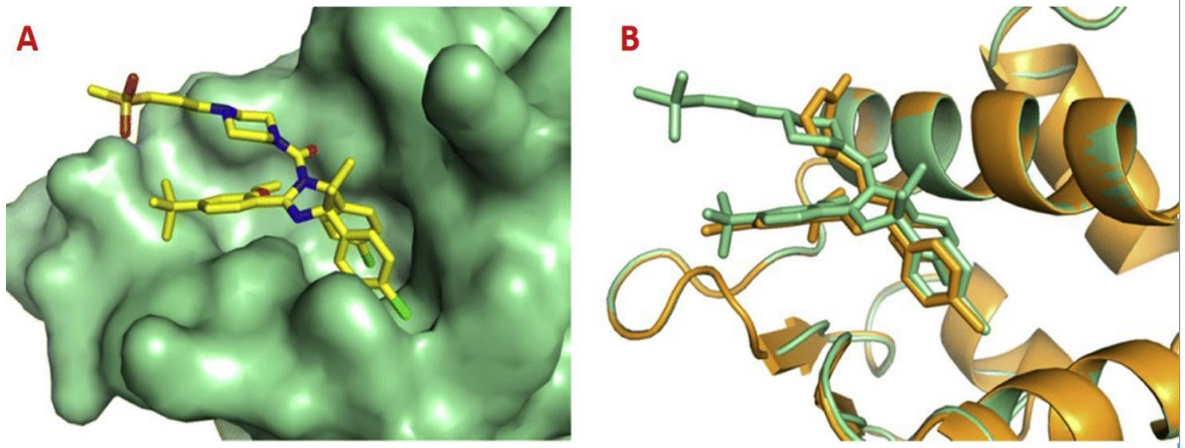


Nutlin-3a

Vassilev, L. T. (2004). ***In Vivo Activation of the p53 Pathway by Small-Molecule Antagonists of MDM2***. *Science, 303(5659), 844–848.* doi:10.1126/science.1092472

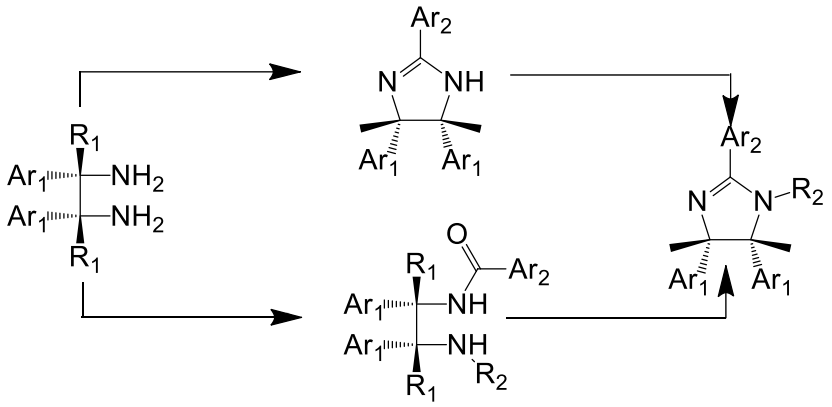
Disadvantages of cis-imidazoline inhibitors:
Low water solubility

The purpose of the work:
Synthesis of new cis-imidazoline inhibitors with increased water solubility and resistance to oxidation.

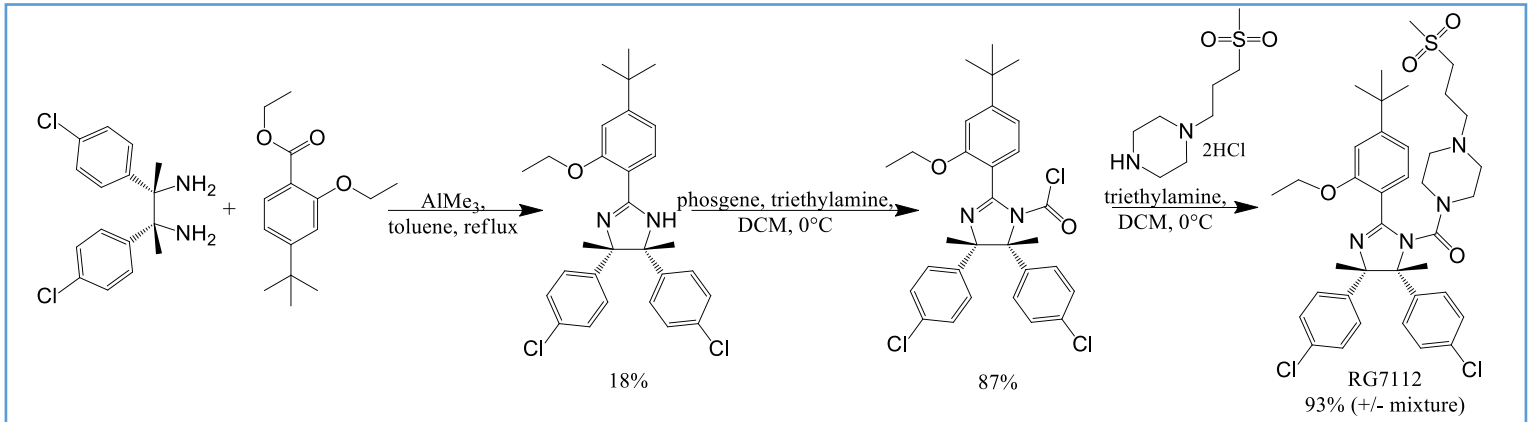


The crystal structure of MDM2 (shown by the green surface) in complex with RG7112 (yellow, PDB code: 4IPF). (B) Superposition of MDM2 crystal structures in complex with RG7112 (green) and Nutlin-3a (gold, PDB code: 4J3E).

№4. Synthetic approach (literary)

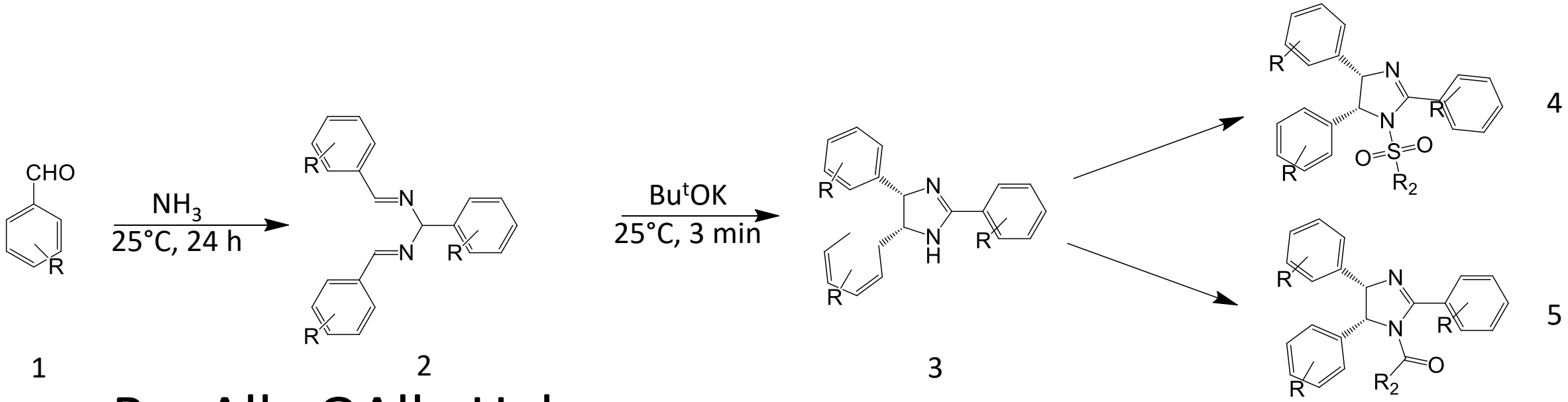


approaches to the synthesis of p53/mdm2 imidazoline inhibitors

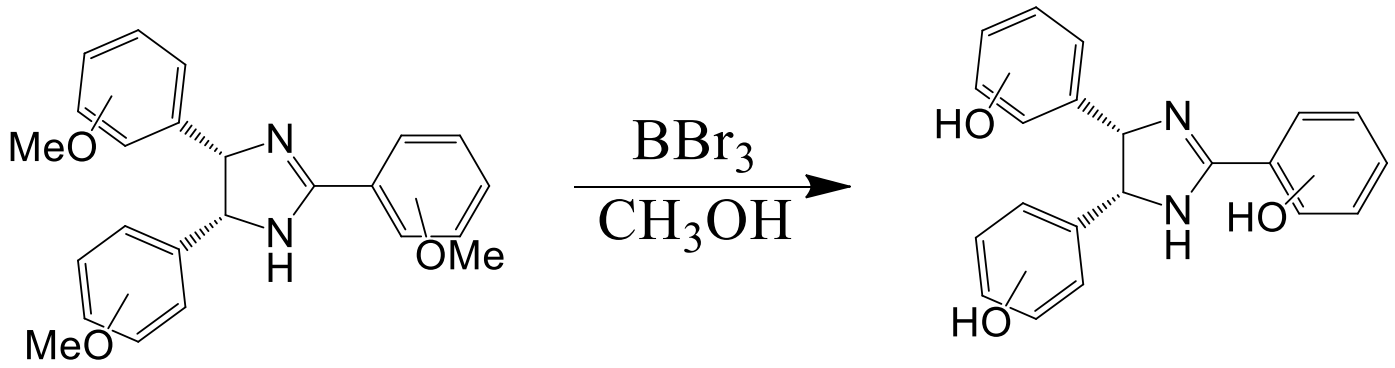


original synthesis of RG7112 inhibitor (Vu, B.; Wovkulich, P.; Pizzolato, G.; Lovey, A.; Ding, Q.; Jiang, N.; Liu, J.J.; Zhao, C.; Glenn, K.; Wen, Y.; et al. ***Discovery of RG7112: A Small-Molecule MDM2 Inhibitor in Clinical Development****. ACS Medicinal Chemistry Letters 2013, 4, 466–469, doi:10.1021/ml4000657)*

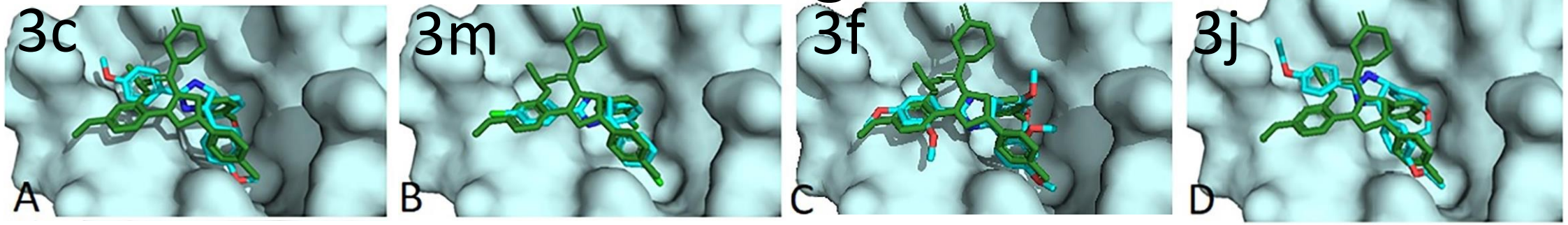
№5. The synthetic approach of this work



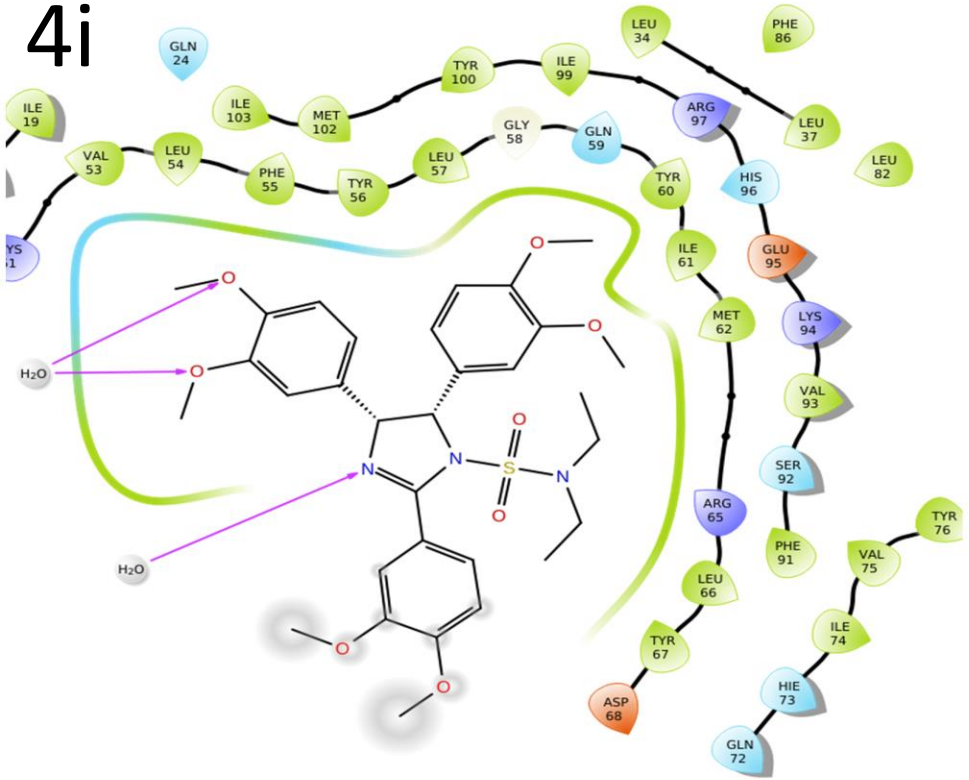
R = Alk, OAlk, Hal



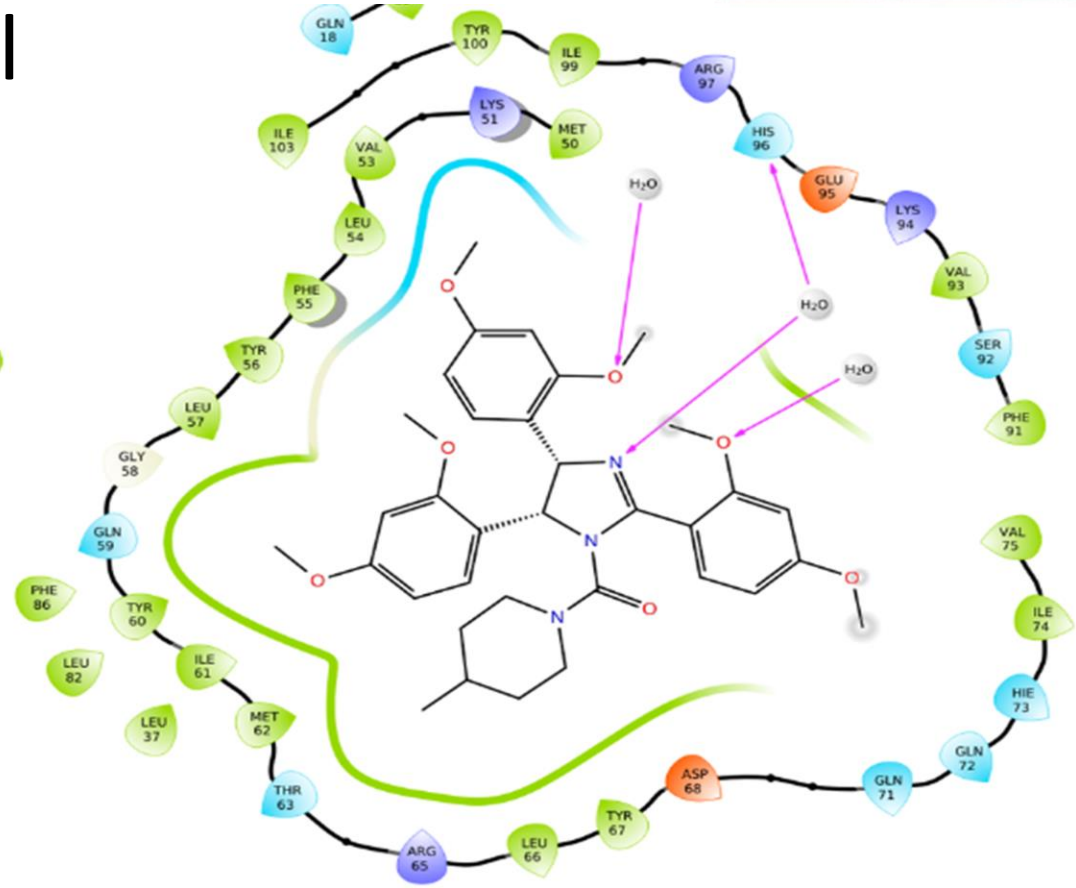
№6. Molecular modeling



4i

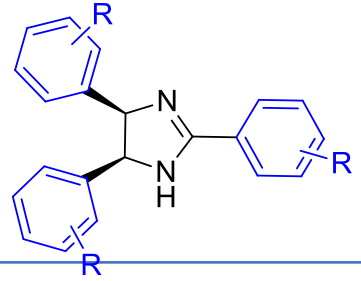


5l



Charged (negative)
Charged (positive)
Glycine
Hydrophobic
Polar
Water
H-bond
Pi-Pi stacking
Solvent exposure

№7. Cytotoxicity (series №3)



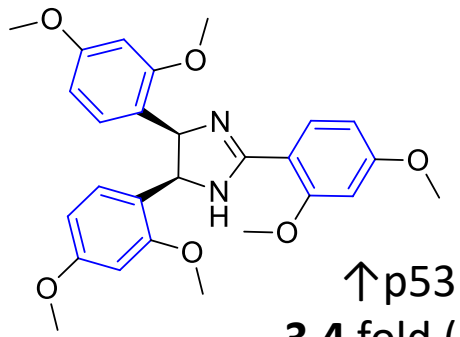
№	Compound	R	A549 (IC50, μM)	№	Compound	R	A549 (IC50, μM)
1	3a	2-MeO	27.36±0.79	**16**	3p	4-Br	9.05±0.20
2	3b	3-MeO	64.31±1.04	**17**	3q	4-F	107.83±4.65
3	3c	4-MeO	43.90±1.87	**18**	3r	3,5-diMeO	-[2]
4	3d	2,3-diMeO	16.18±0.29	**19**	3s	4-OH	n.a.[1]
5	3e	2-EtO,3-MeO	24.26±2.10	**20**	3t	4-OH(R^1),4-MeO(R^2)[3]	n.a.[1]
6	3f	2,4-diMeO	9.32±0.47	**21**	3u	3-OH	310.60±16.67
7	3g	3,4-diMeO	84.69±0.76	**22**	3v	2,5-diOH	n.a.[1]
8	3h	2,5-diMeO	21.42±1.07	**23**	3w	3,4,5-trisHO	-[2]
9	3i	3,4,5-trisMeO	n.a.[1]	**24**	3x	4-Me	-[2]
10	3j	4-EtO	13.26±0.37	**25**	3y	3-Me	-[2]
11	3k	3-MeO,4-EtO	67.49±0.04	**26**	3z	2-Me	-[2]
12	3l	2-Cl	10.68±0.18	**27**	3aa	4-Et	-[2]
13	3m	4-Cl	20.25±1.88	**28**	3ab	4-iPr	-[2]
14	3n	2,4-diCl	164.41±13.74	**29**	3ac	2,4,5-trisMeO	-[2]
15	3o	3,4-diCl	13.72±1.02	**30**	3ad	2,3,4-trisMeO	-[2]
					Nutlin-3a		15.12[15]

1 n.a. lack of activity of this type

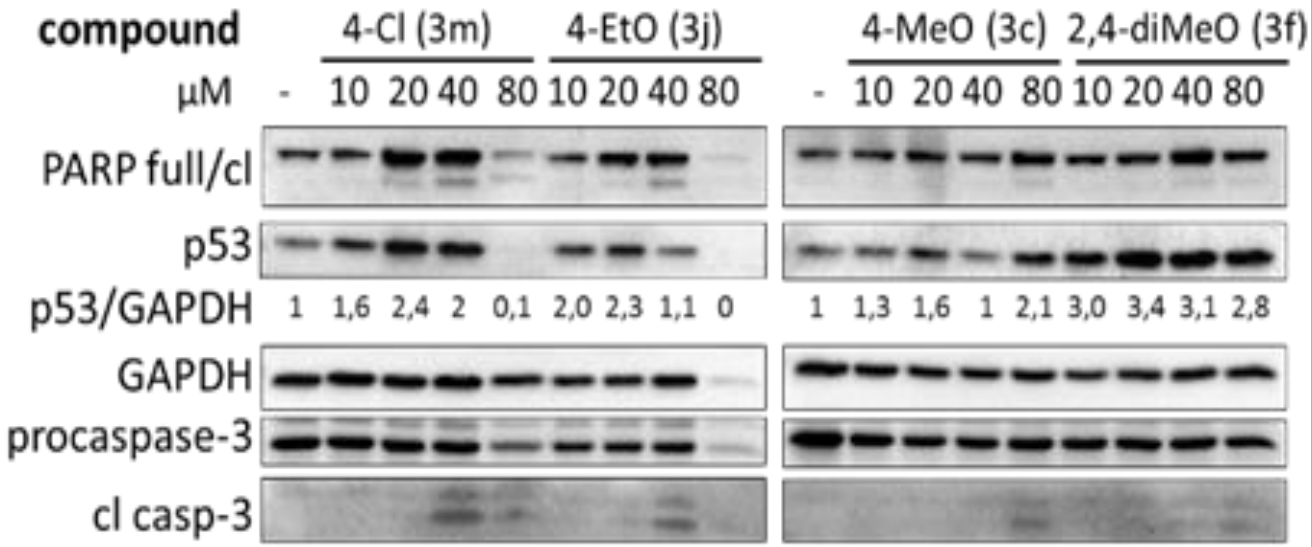
2 tests were not carried out

Bazanov, D.R.; Pervushin, N. V.; Savitskaya, V.Yu.; Anikina, L. V.; Proskurnina, M. V.; Lozinskaya, N.A.; Kopeina, G.S. ***2,4,5-Tris(Alkoxyaryl)Imidazoline Derivatives as Potent Scaffold for Novel P53-MDM2 Interaction Inhibitors: Design, Synthesis, and Biological Evaluation.*** *Bioorganic & Medicinal Chemistry Letters* ***2019****, 29, 2364–2368, doi:10.1016/j.bmcl.2019.06.007.*

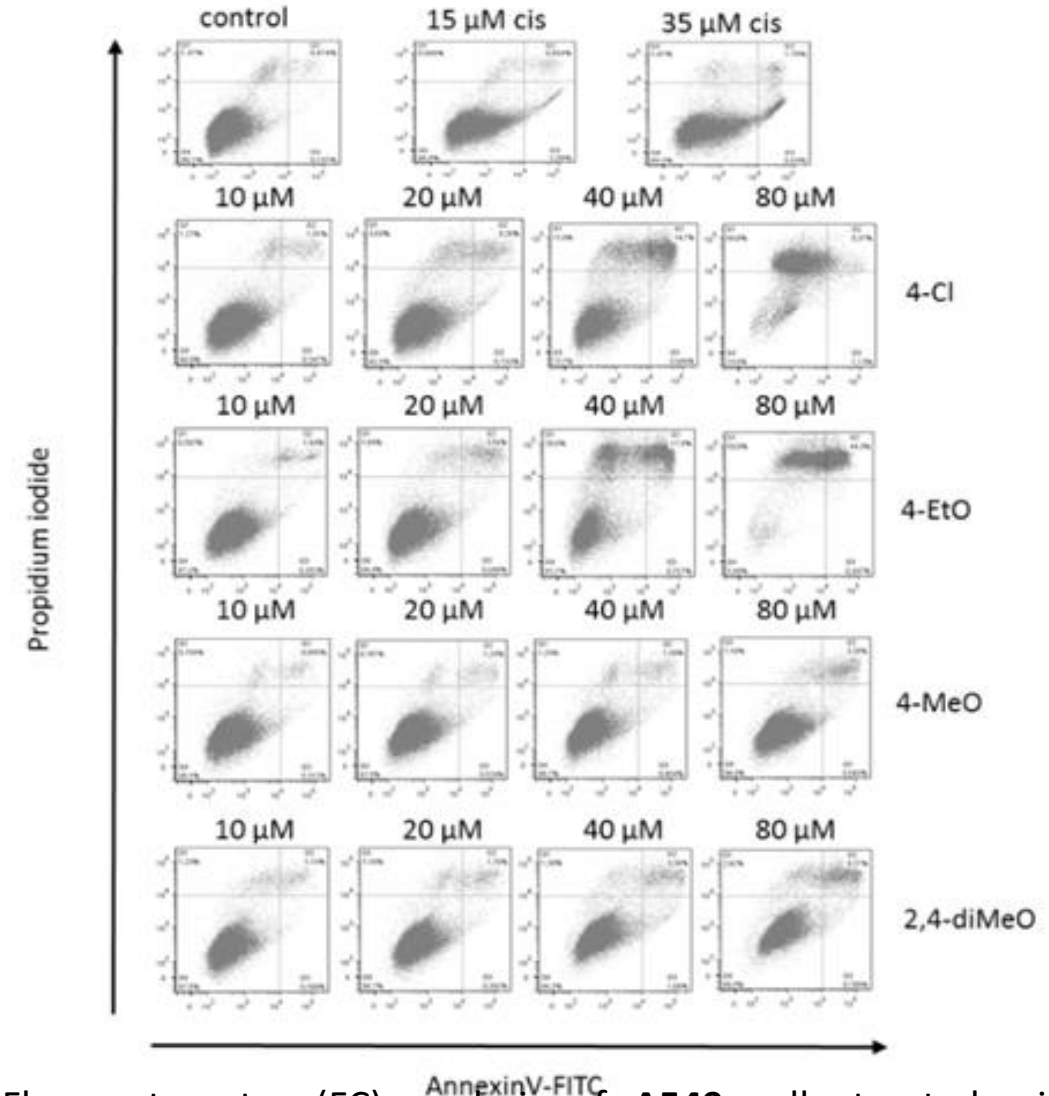
№8. Biological activity (series №3)



↑p53
3.4 fold (3f)



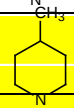
Western Blot analysis of total cellular lysates from **A549** cells upon treatment with indicated compounds.

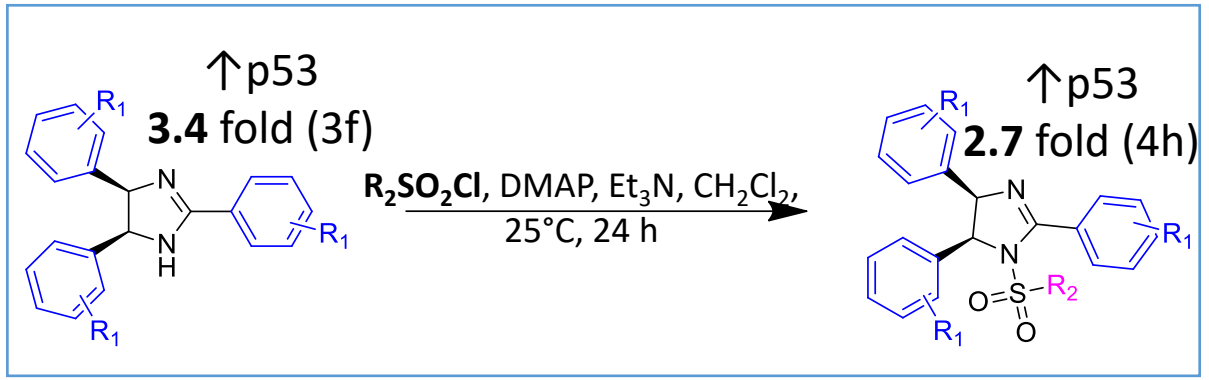


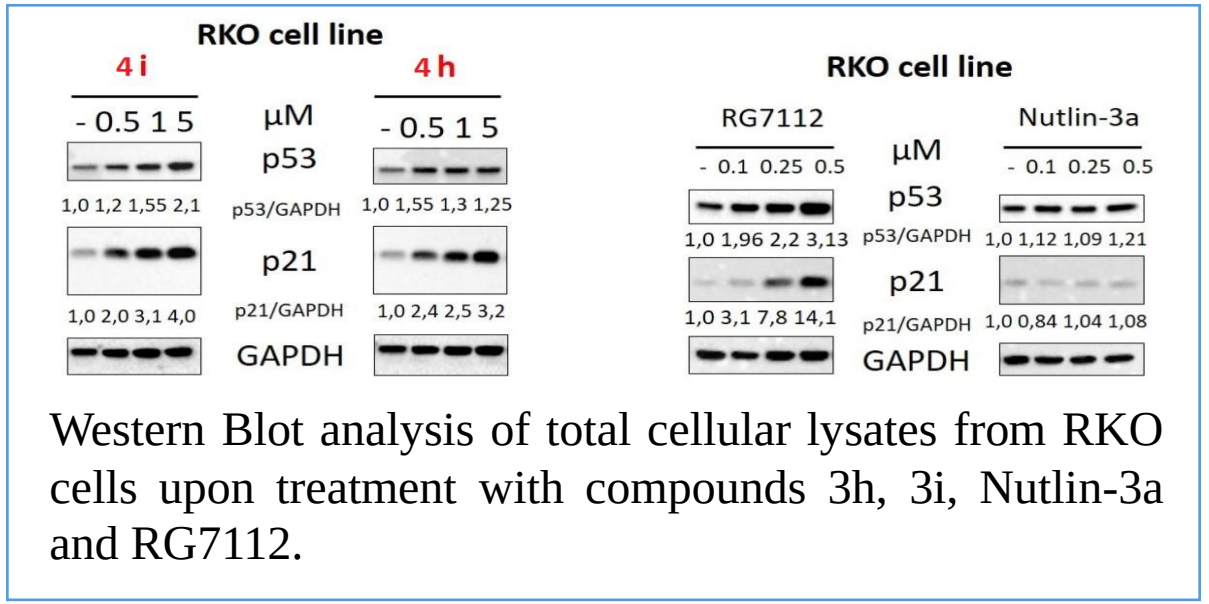
Flow cytometry (FC) analysis of **A549** cells treated with cisplatin, 3c, 3f, 3j and 3m.

Bazanov, D.R.; Pervushin, N. V.; Savitskaya, V.Yu.; Anikina, L. V.; Proskurnina, M. V.; Lozinskaya, N.A.; Kopeina, G.S. ***2,4,5-Tris(Alkoxyaryl)Imidazoline Derivatives as Potent Scaffold for Novel P53-MDM2 Interaction Inhibitors: Design, Synthesis, and Biological Evaluation.*** *Bioorganic & Medicinal Chemistry Letters* ***2019****, 29, 2364–2368, doi:10.1016/j.bmcl.2019.06.007.*

№9. Synthesis and biological activity of sulfonamide derivatives (series №4)

№	Compound	R1	R2	Yield, %	↑ p53
1	**4a**	4-MeO	Ts	85	-
2	**4b**	4-MeO		78	-
3	**4c**	4-MeO		76	-
4	**4d**	2,4-diMeO	Ts	73	-
5	**4e**	2,4-diMeO		36	-
6	**4f**	3,4-diMeO	Ts	36	0,7
7	**4g**	3,4-diMe		31	1,5
8	**4h**	3,4-diMeO		35	2,7
9	**4i**	3,4-diMeO	Et_2N	37	2,3
10	**4j**	3,4-diMeO		27	0,8
11	**4k**	2,5-diMeO		81	-
12	**4l**	2,5-diMeO		76	-

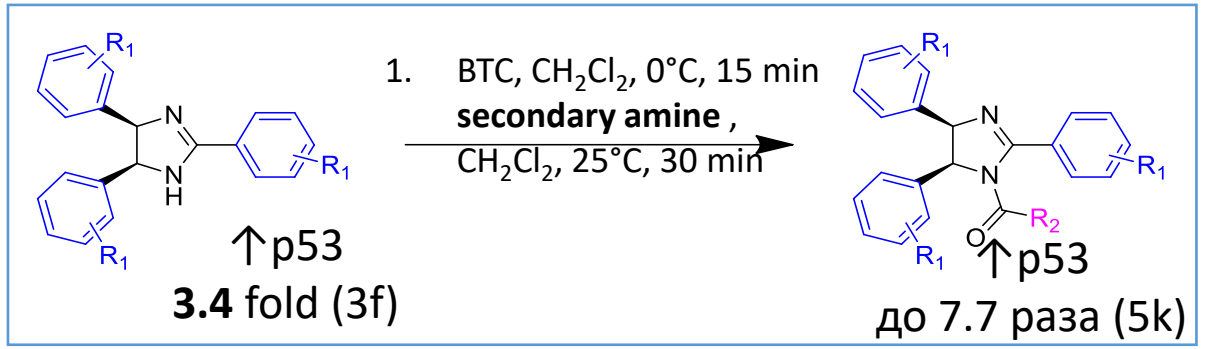




Western Blot analysis of total cellular lysates from RKO cells upon treatment with compounds 3h, 3i, Nutlin-3a and RG7112.

Bazanov, D.R.; Pervushin, N. V; Savin, E. V; Tsymliakov, M.D.; Maksutova, A.I.; Sosonyuk, S.E.; Kopeina, G.S.; Lozinskaya, N.A. **Sulfonamide Derivatives of Cis -Imidazolines as Potent P53-MDM2 / MDMX Protein-Protein Interaction Inhibitors.** *Medicinal Chemistry Research* **2021**, doi:10.1007/s00044-021-02802-w.

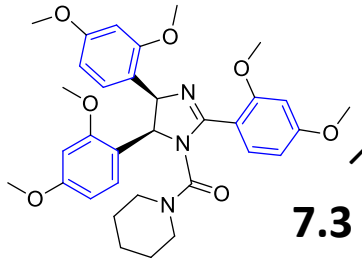
№10. Synthesis and biological activity of carbamides (series №5)



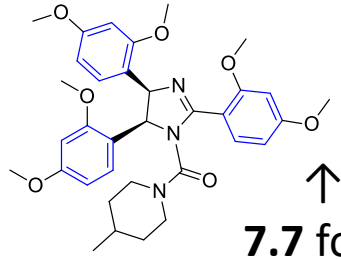
№	Compound	R_1	R_2	IC50, μM A549	№	Compound	R_1	R_2	IC50, μM A549	№	Compound	R_1	R_2	IC50, μM A549
1	**5a**	4-MeO		58.2±9.2	10	**5j**	2,4-diMeO		105,7±20,2	19	**5s**	3,4-diMeO		102.1±50
2	**5b**	4-MeO		165±100	11	**5k**	2,4-diMeO		30±2,5	20	**5t**	3,4-diMeO		>200
3	**5c**	4-MeO		53.9±7	12	**5l**	2,4-diMeO		17,8±2,1	21	**5u**	3,5-diMeO		24,6±6,3
4	**5d**	4-MeO		38±5	13	**5m**	2,4-diMeO		21±3	22	**5v**	3,5-diMeO		93.6±20
5	**5e**	4-MeO		124±60	14	**5n**	3,4-diMeO		105.2±25	23	**5w**	3,5-diMeO		104,3±36
6	**5f**	4-MeO		38,8±4,5	15	**5o**	3,4-diMeO		123.4±41.5	24	**5x**	4-Cl		16,2±3,1
7	**5g**	4-MeO		89,8±7,5	16	**5p**	3,4-diMeO		104.4±50.5	25	**5y**	2,4-diCl		25,2±8,2
8	**5h**	2-MeO		60,4±26,3	17	**5q**	3,4-diMeO		123.1±50	26	**5z**	2,4-diCl		18,7±2,5
9	**5i**	2,4-diMeO		35,2±6,4	18	**5r**	3,4-diMeO		-	27	**5aa**	2,4-diCl		86,6±45,4

Bazanov, D.R.; Pervushin, N.V.; Savin, E.V. Michael D. Tsymliakov, Anita I. Maksutova, Victoria Yu. Savitskaya, Sergey E. Sosonyuk, Yulia A. Gracheva, Michael Yu. Seliverstov, Natalia A. Lozinskaya and Gelina S. Kopeina. **Synthetic design and biological evaluation of new p53-MDM2 interaction inhibitors based on imidazoline core.** *Pharmaceuticals* **2022**, *15*, 4. https://doi.org/10.3390/ph15040444

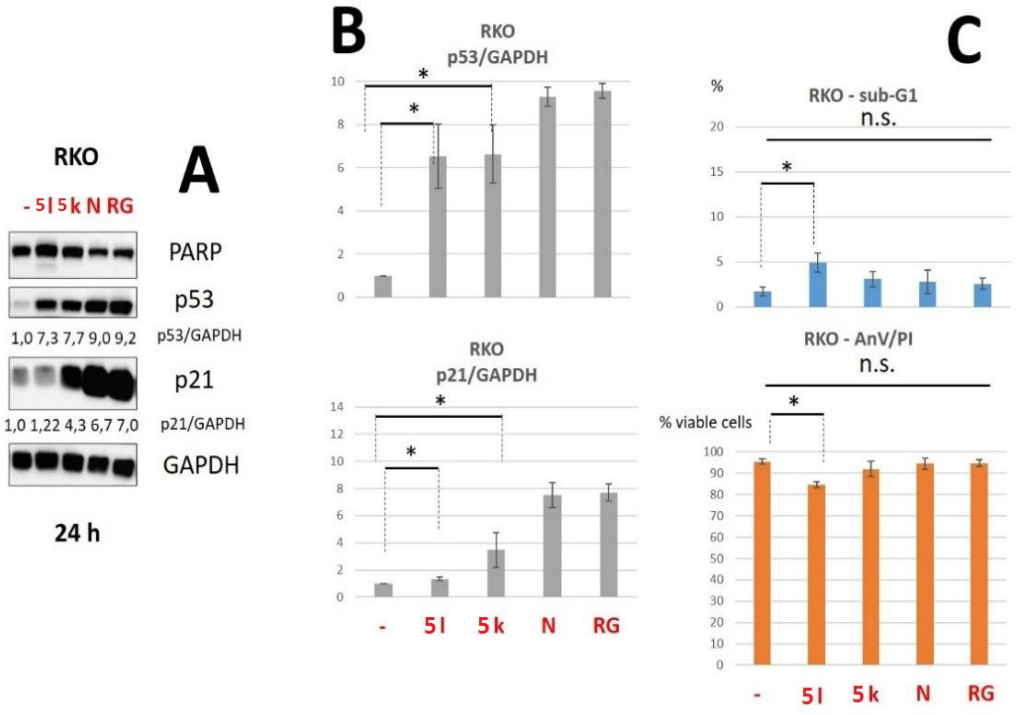
№11. Synthesis and biological activity of carbamides (series №5)



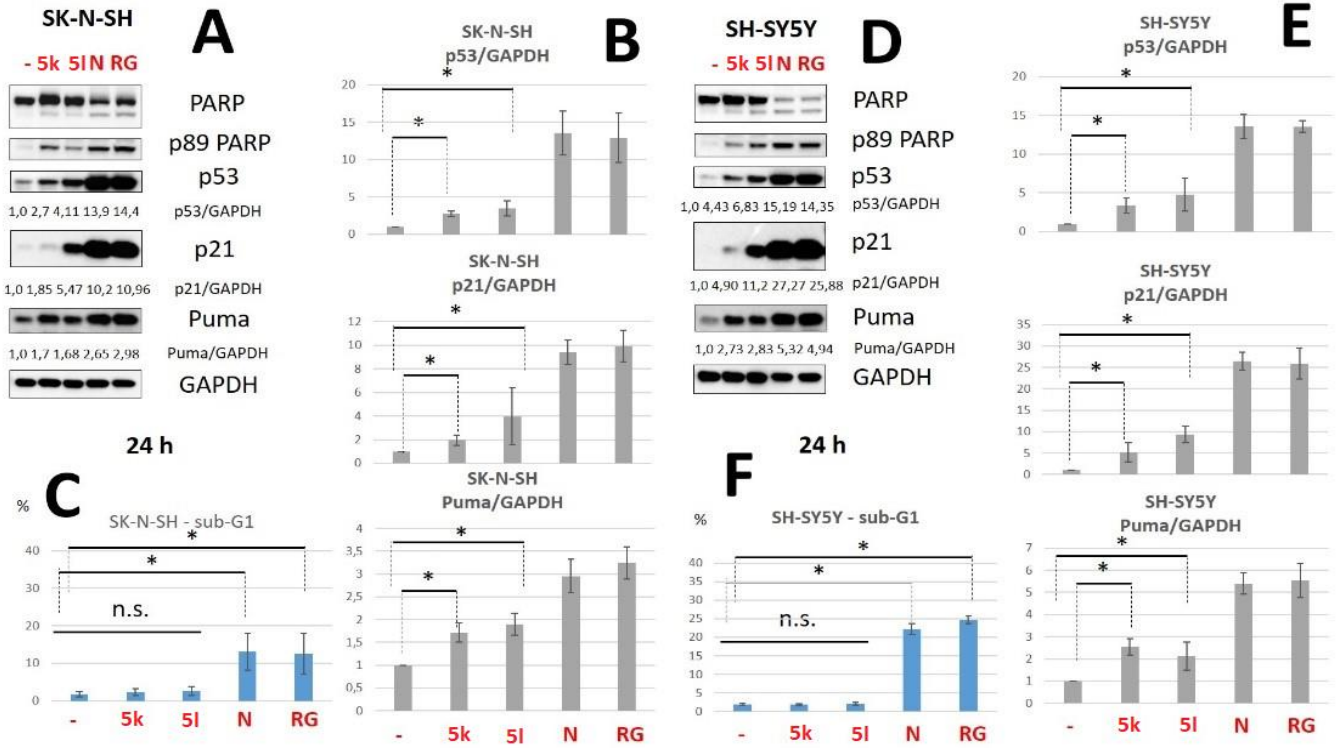
↑p53
7.3 fold (5l)



↑p53
7.7 fold (5k)



(A). Western Blot analysis of total cellular lysates from RKO cells upon the treatment with compounds 2l, 2k (both—20 μM), Nutlin-3a (10 μM) and RG7388 (5 μM). (B)—Densitometric analysis of p53 bands normalized to GAPDH. Data are presented as mean +/− SD from three independent experiments. (C)—The histograms of flow cytometry (FC) analysis data for RKO cells: sub-G1 assay (up), %—percent of Sub-G1 population and Annexin V-FITC/PI staining (below), % viable cells—cells negative for both Annexin V-FITC and propidium iodide (PI).



Western Blot analysis of total cellular lysates from SK-N-SH (A) and SH-SY5Y (D) cells upon the treatment with compounds 2l, 2k (both—20 μM), Nutlin-3a (10 μM) and RG7388 (5 μM). (B,E)—Densitometric analysis of p53, p21 and Puma bands normalized to GAPDH in SK-N-SH (B) and SH-SY5Y (E) cells. Data are presented as mean +/− SD from three independent experiments. (C,F)—The histogram of flow cytometry (FC) analysis data for SK-N-SH cells using sub-G1 assay, %—percent of Sub-G1 population.

Bazanov, D.R.; Pervushin, N.V.; Savin, E.V. Michael D. Tsymliakov, Anita I. Maksutova, Victoria Yu. Savitskaya, Sergey E. Sosonyuk, Yulia A. Gracheva, Michael Yu. Seliverstov, Natalia A. Lozinskaya and Gelina S. Kopeina. **Synthetic design and biological evaluation of new p53-MDM2 interaction inhibitors based on imidazoline core.** *Pharmaceuticals* **2022**, *15*, 4. https://doi.org/10.3390/ph15040444

12. Conclusions

1. A series of 2,4,5-triarylimidazolines was synthesized, the cytotoxicity of the derivatives obtained was tested, and the compound that most effectively stabilizes the level of p53 in tumor cells was determined.
2. Modification of 2,4,5-triarylimidazolines with sulfamoyl chloride derivatives was carried out, their biological activity was evaluated by the ability to stabilize the p53 protein.
3. Modification of 2,4,5-triarylimidazolines by derivatives of secondary amines and BTC was carried out. A leader compound has been determined that stabilizes the p53 level by more than 7 times compared to the control. Activity was confirmed on five cell lines.