MPGI STRATEGY IMPROVES AQUEOUS SOLUBILITY IF COLCHICINE SITE INHIBITORS OF TUBULIN POLYMERIZATION

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INTRODUCTION AND OBJECTIVES

Antimitotic agents that bind to tubulin at colchicine site have serious drawback because of their low aqueous solubility. In this work we have design and synthesized compounds in which the classical trimethoxyphenyl moiety of colchicine site antimitotic ligands is replaced by substituted anilines.

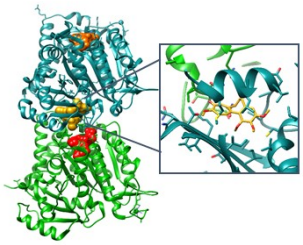
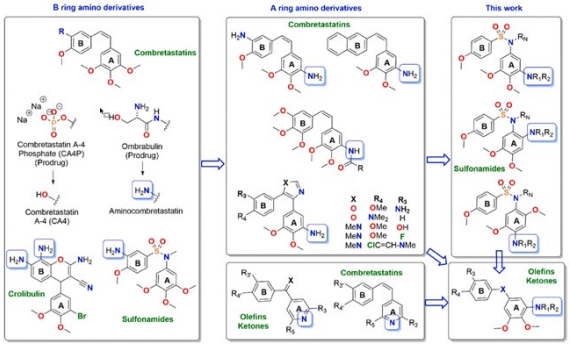
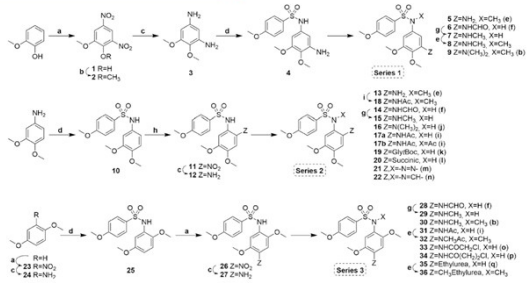


Figure 1. Left.: Schematic structure of Colchicine site in tubulin. Right:X-Ray image tubulin-colchicine complex (PDB ID: 4O2B).

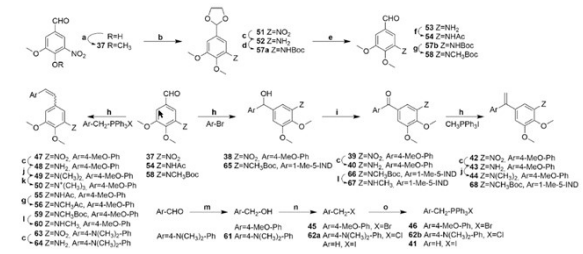


Scheme 1. Design of new A-ring amino derivatives.

CHEMICAL SYNTHESIS



Scheme 2. Chemical synthesis of compounds in series 1–3. Reagents, conditions, and yields: (a) HNO_3, AcOH, 0 °C, 1–4 h, 90–94 %. (b) $(CH_3)_2SO_4$, K_2CO_3, acetone, reflux, 12–48 h, 15–98 %. (c) H_2, Pd (C), EtOAc, 48–72 h, 82–99 %. (d) 4-Methoxybenzenesulfonyl chloride, pyridine, CH_2Cl_2, 4–12 h, 73–99 %. (e) CH_3I, KOH, CH_3CN, 24 h, 52–98 %. (f) Formic acid, pyridine, CH_2Cl_2, 48–72 h, 67–89 %. (g) Trichloroacetic acid, $NaBH_4$, dry THF, 0 °C, 24–48 h, 34–93 %. (h) *tert*-Butyl nitrite, CH_3CN, 45 °C, 24 h, 95 %. (i) Acetic anhydride, pyridine, CH_2Cl_2, 30 min 24 h, 32–90 %. (j) Paraformaldehyde, $NaBH_3CN$, AcOH, MeOH, 96 h, 40 %. (k) Succinic anhydride, pyridine, CH_2Cl_2, 24 h, 59 % (l) (*tert*-butoxycarbonyl)glycine, EDCI, 4-DMAP, CH_2Cl_2, 24 h, 30 %. (m) *tert*-Butyl nitrite, CH_3CN, H_2O, AcOH, 0 °C, 24 h, 29 %. (n) Triethyl orthoformate, CH_3CN, reflux, 12 h, 97 %. (o) 2-Chloroacetyl chloride, CH_2Cl_2, 12 h, 87 %. (p) 3-Chloropropanoyl chloride, CH_2Cl_2, 12 h, 48 %. (q) Ethyl isocyanate, CH_2Cl_2, 6 h, 73 %.

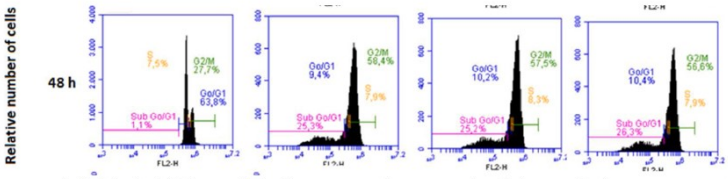


Scheme 3. Chemical synthesis of compounds in series 4. Reagents, conditions, and yields: (a) $(CH_3)_2SO_4$, K_2CO_3, acetone, reflux, 48 h, 81 %. (b) $HO(CH_2)_2OH$, TsOH, toluene, Dean-Stark, 120 °C, 24 h, 96 %. (c) Zn powder, AcOH, CH_2Cl_2, 0 °C, 4–24 h, 69–98 %. (d) Boc_2O, THF, reflux, 48 h, 81 %. (e) HCl/H_2O, MeOH, 4 h, 97 %. (f) Ac_2O, pyridine, CH_2Cl_2, 2 h, 72 %. (g) CH_3I, NaH, THF, 15 h, 75–90 %. (h) *n*BuLi, dry THF, −78 °C to room temperature, 24 h, 47–97 %. (i) PDC, CH_2Cl_2, 0 °C, 4 h, 37–83 %. (j) CH_3I, acetone, reflux, 24 h, 88–97 %. (k) CH_3I, $AgNO_3$, acetone, 24 h, 49 %. (l) TMSCl, MeOH, 24 h, 56–88 %. (m) $NaBH_4$, MeOH, 2 h, 95 %. (n) X = Br: PBr_3, Et_2O, -40 °C, 4 h, 98 %; X = Cl: HCl(g), CH_2Cl_2. (o) PPh_3, toluene, 15–24 h, 60–91 %. Abbreviations: 4-MeO-Ph = 4-methoxyphenyl; 4-$N(CH_3)_2$-Ph = 4-dimethylaminophenyl; 1-Me-5-IND = *N*-methyl-5-indolyl.

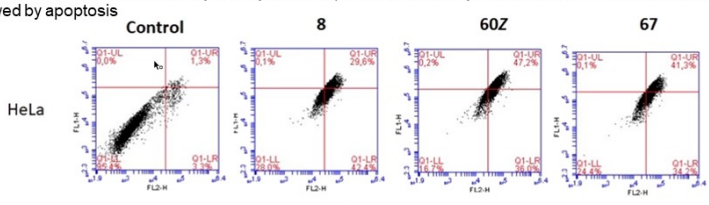
BIOLOGICAL RESULTS

Comp	Antiproliferative activity IC_{50} (nM)[a] HeLa	MCF7	HT-29	HT-29 Verap[b]	IC_{50} TPI (μM)[a]	Solub (μg/mL)
4	>1000	>1000	>1000	n.d.[d]	>10	243
5	>1000	>1000	>1000	n.d.	>10	47
6	>1000	>1000	>1000	n.d.	>10	16
7	411	530	>1000	n.d.	>10	60
8	71	71	410	130	32.6	164
9	337	390	662	450	>10	71
10	>1000	>1000	>1000	n.d.	>10	02
12	>1000	>1000	>1000	n.d.	>10	460
13	>1000	>1000	>1000	n.d.	>10	n.d.
14	>1000	>1000	>1000	n.d.	>10	n.d.
15	>1000	>1000	>1000	n.d.	>10	n.d.
16	630	n.d.	>1000	n.d.	>10	30
17a	>1000	>1000	>1000	n.d.	>10	454
17b	>1000	>1000	>1000	n.d.	>10	400
18	>1000	>1000	>1000	n.d.	>10	n.d.
19	>1000	>1000	>1000	n.d.	>10	1172
20	>1000	>1000	>1000	n.d.	>10	255
21	>1000	>1000	>1000	n.d.	>10	220
22	>1000	>1000	>1000	n.d.	>10	4409
25	227	350	187	107	>10	13
27	>1000	>1000	>1000	n.d.	>10	51
28	>1000	>1000	>1000	n.d.	>10	44
29	697	>1000	>1000	n.d.	>10	109
30	1070	>1000	>1000	n.d.	>10	20
31	>1000	>1000	>1000	n.d.	>10	n.d.
32	>1000	>1000	>1000	n.d.	>10	n.d.
33	>1000	>1000	>1000	n.d.	>10	n.d.
34	>1000	>1000	>1000	n.d.	>10	n.d.
35	>1000	>1000	>1000	n.d.	>10	n.d.
36	>1000	>1000	>1000	n.d.	n.d.	n.d.
40	203	413	>1000	n.d.	>5	110
43	190	65	400	463	2.5	102
44	397	537	2000	600	6.0	n.d.
40Z	190	63	650	613	6.2	n.d.
49Z	260	57	233	240	1.1	71
50Z	4070	973	>1000	n.d.	>5	1000
55Z	810	677	5500	907	>5	40
56Z	>1000	>1000	>1000	n.d.	>5	44
59Z	650	507	>1000	n.d.	>10	n.d.
60Z	13	4	309	73	0.8	52
64Z	400	753	>1000	n.d.	>5	6.7
67	13	12	303	430	1.2	10
68	>1000	>1000	>1000	n.d.	>5	6.3
CA-4	2	1	305	327	3	1
ABT-751	303	100	213	250	4.4	40

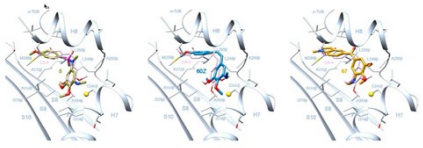
The amino-substituted analogs and particularly the methyl-amino ones are potent tubulin polymerization inhibitors and cytotoxic compounds against several human cancer cell lines while significantly improving the intrinsic aqueous solubility. The compounds are mostly not substrates of the MDR pump, one of the main resistance mechanisms of cancer cells in the clinic.



In agreement with tubulin inhibitory activity, the compounds cause early mitotic arrest in human cancer cell lines followed by apoptosis



Molecular modeling studies support binding at the colchicine site. Anilines located at the A ring subpocket hide the polar hydrogen on the amino groups through intramolecular hydrogen bonds to their neighboring methoxyl oxygens.



CONCLUSIONS

Hiding of polar groups in apolar environments (tubulin) while exposing them in polar solvents (water) to improve the aqueous solubility represents a new successful application of the masked polar group incorporation (MPGI) strategy towards new colchicine site antimitotic agents with improved solubility and with favorable calculated clogP and TPSA values, deemed as predictors of good absorption and low toxicity, as well.

The methylamino substituted compounds combine a nanomolar cytotoxic potency with improved water solubilities and are promising candidates for further studies.

Myriam González, Younes Ellahioui, Laura Gallego, Alba Vicente-Blázquez, Raquel Álvarez, Manuel Medarde, Rafael Peláez, Novel amino analogs of the trimethoxyphenyl ring in potent colchicine site ligands improve solubility by the masked polar group incorporation (MPGI) strategy, *Bioorganic Chemistry*, Volume 131,2023,106282,

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