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New chalcone derivatives with suitable drug-like lipophilicity targeting mitosis

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#Authors contributed equally to this work

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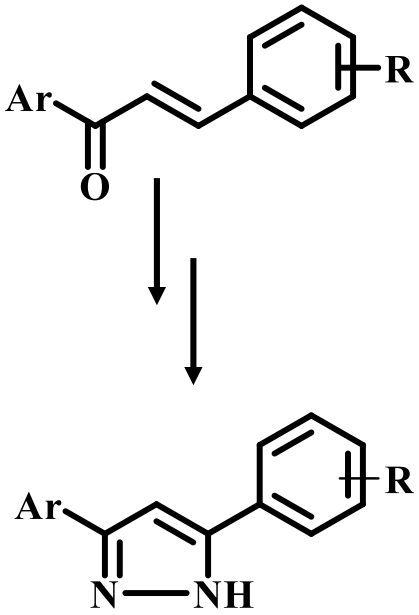
New chalcone derivatives with suitable drug-like lipophilicity targeting mitosis

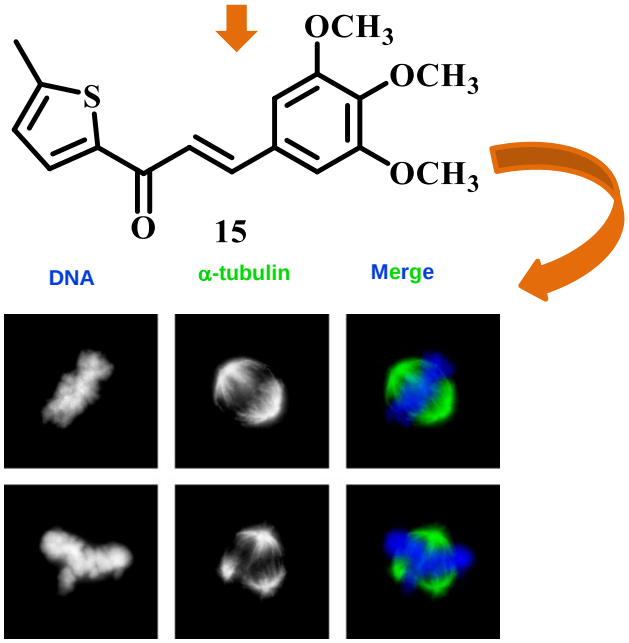
1. Synthesis



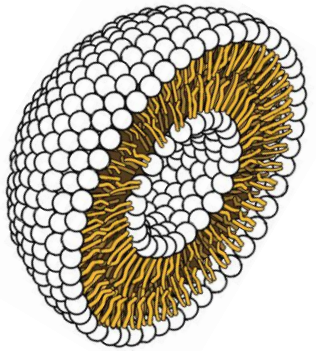
2. Human Tumor Cell lines

Compounds **3, 5, 9, 11, 15-19** $GI_{50} < 10$ µM

15-17 exhibited **antimitotic activity**



3. Lipophilicity evaluation



Most potent compounds ($GI_{50} < 8$ µM)

3.30 < lop Kp < 3.68

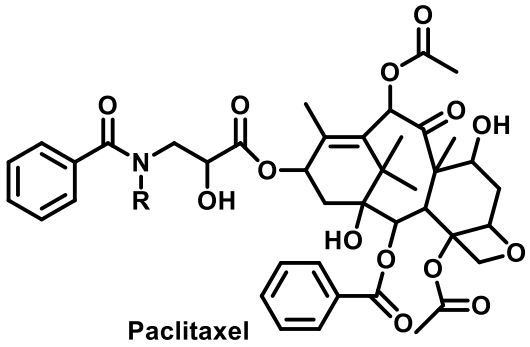
Abstract:
Chalcones are natural flavonoid precursors that have been reported for their wide range of biological activities, namely antitumor [1-2]. In addition, the presence of an α,β-unsaturated ketone moiety makes these compounds a valuable chemical substrate for the synthesis of bioactive derivatives, such as pyrazoles [3]. Our research group has reported two synthetic chalcone derivatives with antimitotic effect [4-5]. Hence, in continuation of our efforts to obtain new chalcone derivatives with improved antitumor and antimitotic activity, a small library of chalcone derivatives, including pyrazole and α,β-epoxide, was synthesized and evaluated for their cell growth inhibitory activity in three human tumor cell lines. Additionally, their lipophilicity using liposomes as a biomimetic membrane model was determined. From this work, nine chalcones showing suitable drug-like lipophilicity with antimitotic effect were identified. Moreover, one of the compounds was able to enhance chemosensitivity of tumor cells to paclitaxel in NCI-H460 cells.

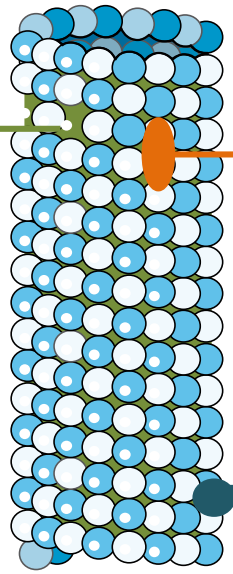
Keywords: Chalcone derivatives; lipophilicity; mitosis

Introduction: Microtubules Targeting Agents (MTAs)

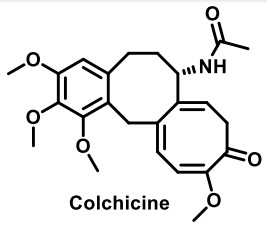
Stabilizers | **Destabilizers**

Taxane binding site

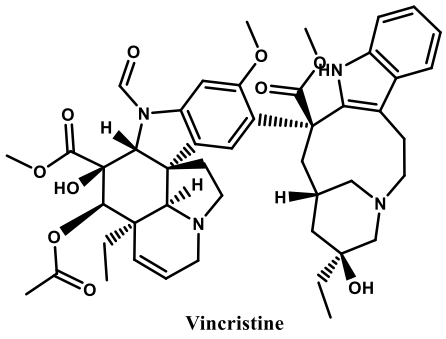




Colchicine binding site



Vinca alkaloids binding site



Disadvantages of MTAs

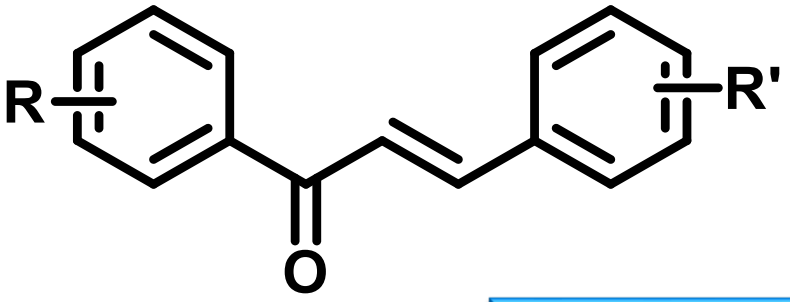
- Hematopoietic toxicity
- Neurologic toxicity
- Drug resistence

New Antimitotic agents

Introduction: Chalcones

Biological Activities

- Anti-inflammatory
- Anti-tuberculosis
- Antidiabetic
- Antimicrobial
- Antioxidant

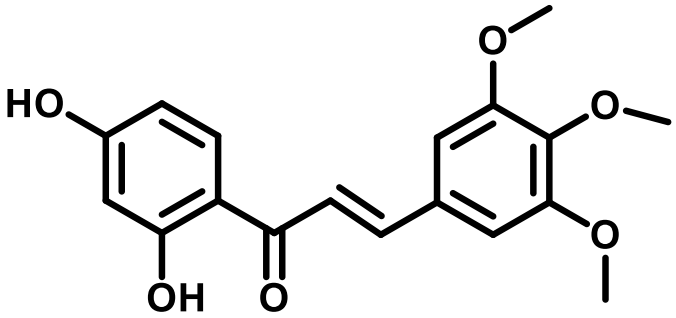


- Antitumor
  - **Molecular targets**
    - p53/MDM2 interaction
    - Sex hormones
    - mTOR pathway
    - NF-Kb pathway
    - Oxireductases
    - ABC transporters
    - Microtubules – Tubulin Polymerization
- Cardiovascular agents
- Antiulcer
- Antimalarial
- Antimicrobial
- Antileishmanial

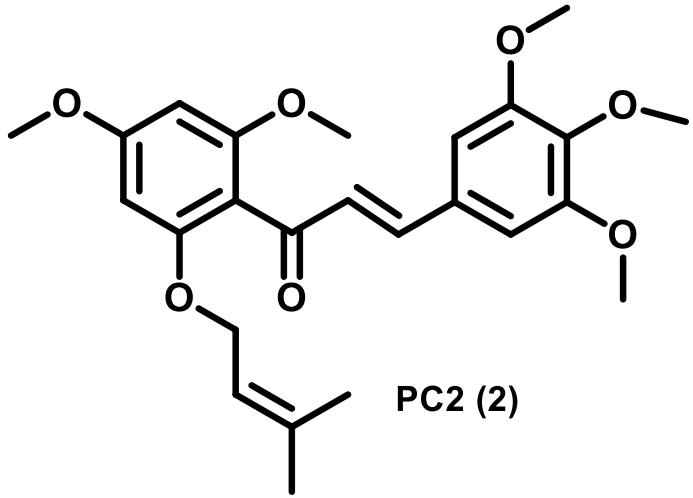
Singh, P.; Anand, A.; Kumar, V. *Eur. J. Med. Chem.* 2014, *85*, 758-777.

Introduction: Chalcones with Antimitotic Effect

Chalcones with antimitotic effect previously reported by our research group:



1



PC2 (2)

Caused abnormal spindle apparatus assembly

Prolonged mitotic arrest followed by cell death

Masawang K. et al., Toxicol. Lett.. 2014, 229, 393-401. Fonseca J. *et al.*, Molecules 2016, 21, 982

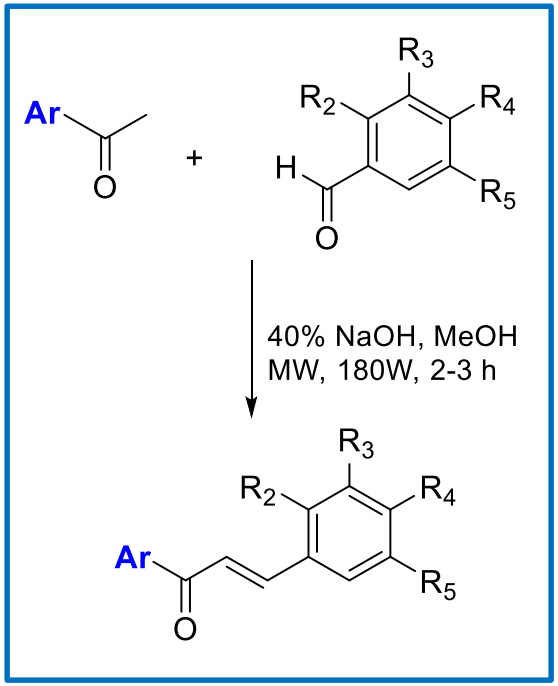
Aims

To obtain new chalcone derivatives with promising antimitotic effect with suitable drug-like lipophilicity

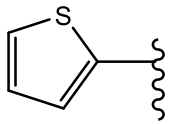
- Synthesis of a small library of chalcones, structure related with **1** and **PC2** (**2**)
- Synthesis of pyrazole derivatives
- Evaluate the growth inhibitory effect of all synthesized chalcone derivatives
- Assess the antimitotic effect of the most promising chalcone derivatives
- Determine lipophilicity of all synthesized chalcone derivatives

Results and Discussion

Synthesis of Chalcones

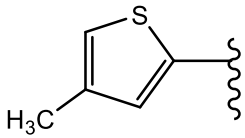


Ar =



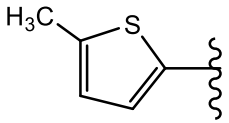
$R_2 = H,\ R_3, R_4, R_5 = OCH_3;$ n = 71%; **3**
$R_3 = H,\ R_2, R_4, R_5 = OCH_3;$ n = 24%; **4**
$R_2, R_4 = H,\ R_3, R_5 = OCH_3;$ n = 44%; **5**
$R_2, R_5 = H,\ R_3, R_4 = OCH_3;$ n = 44%; **6**
$R_2, R_4 = H,\ R_3, R_5 = Cl;$ n = 46%; **7**
$R_2, R_5 = H,\ R_3, R_4 = Cl;$ n = 66%; **8**

Ar =



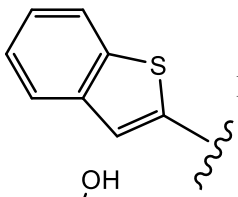
$R_2 = H,\ R_3, R_4, R_5 = OCH_3;$ n = 52%; **9**
$R_3 = H,\ R_2, R_4, R_5 = OCH_3;$ n = 23%; **10**
$R_2, R_4 = H,\ R_3, R_5 = OCH_3;$ n = 46%; **11**
$R_2, R_5 = H,\ R_3, R_4 = OCH_3;$ n = 28%; **12**
$R_2, R_4 = H,\ R_3, R_5 = Cl;$ n = 65%; **13**
$R_2, R_5 = H,\ R_3, R_4 = Cl;$ n = 67%; **14**

Ar =



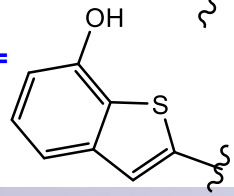
$R_2 = H,\ R_3, R_4, R_5 = OCH_3;$ n = 16%; **15**

Ar =



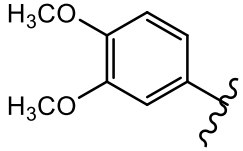
$R_2 = H,\ R_3, R_4, R_5 = OCH_3;$ n = 38 %; **16**

Ar =



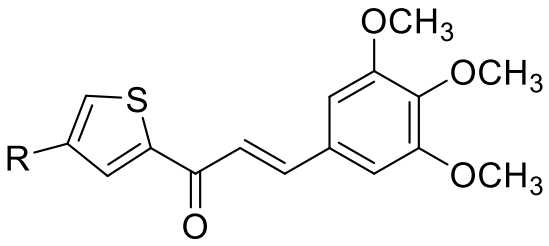
$R_2 = H,\ R_3, R_4, R_5 = OCH_3;$ n = 71%; **17**

Ar =



$R_2 = H,\ R_3, R_4, R_5 = OCH_3;$ n = 61%; **18**
$R_3 = H,\ R_2, R_4, R_5 = OCH_3;$ n = 54%; **19**
$R_2, R_4 = H,\ R_3, R_5 = OCH_3;$ n = 67%; **20**
$R_2, R_5 = H,\ R_3, R_4 = OCH_3;$ n = 28%; **21**
$R_2, R_4 = H,\ R_3, R_5 = Cl;$ n = 82%; **22**
$R_2, R_5 = H,\ R_3, R_4 = Cl;$ n = 80%; **23**

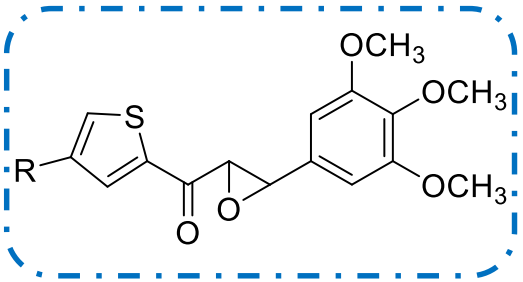
Synthesis of Pyrazole Derivatives



3 R= H
9 R= CH_3

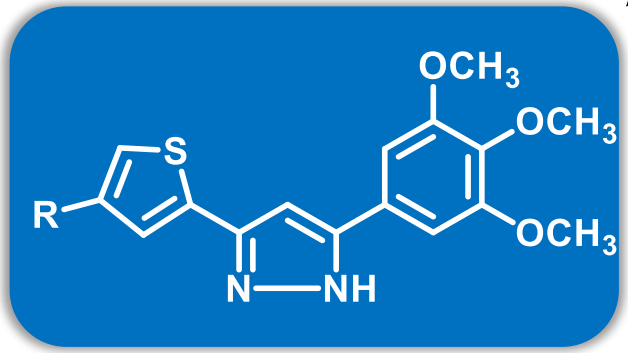
H_2O_2, 5% NaOH,
CH_3COCH_3 : CH_3OH (3:2),
r.t., 2-3 h;

Chalcone epoxide



24 η= 61 %
25 η= 58 %

$NH_2NH_2.H_2O$,
p-toluenesulfonic acid, Xylenes
and dichloromethane,
100 ºC, 3-5 h



26 η= 4 %
27 η= 1 %

Results and Discussion

Evaluation of the Antiproliferative Activity

	GI_{50} (μM)		
	A375-C5	MCF-7	NCI-H460
3	3.63 ± 0.58	5.95 ± 0.88	5.06 ± 0.20
4	11.12 ± 0.96	12.60 ± 2.68	13.62 ± 2.61
5	4.15 ± 0.85	7.70 ± 2.32	7.12 ± 0.20
6	17.77 ± 5.08	23.92 ± 7.18	17.76 ± 2.97
7	5.37 ± 1.47	11.65 ± 4.57	8.34 ± 2.02
8	7.25 ± 2.97	12.12 ± 2.33	8.44 ± 2.13
9	3.21 ± 0.45	3.26 ± 0.11	3.02 ± 0.01
10	6.96 ± 0.65	10.06 ± 3.70	7.48 ± 0.41
11	3.33 ± 1.18	4.28 ± 2.17	4.44 ± 0.87
12	11.27 ± 1.30	10.78 ± 4.44	15.28 ± 2.85
13	7.14 ± 1.87	12.17 ± 2.79	11.85 ± 3.46
14	12.14 ± 1.87	22.54 ± 1.84	15.50 ± 5.66
15	5.70 ± 1.45	5.56 ± 1.51	6.28 ± 0.31

	GI_{50} (μM)		
	A375-C5	MCF-7	NCI-H460
16	6.90 ± 1.10	6.89 ± 0.41	6.61 ± 0.63
17	8.57 ± 1.06	9.75 ± 1.24	8.35 ± 0.31
18	2.89 ± 0.19	3.97 ± 0.82	5.60 ± 1.20
19	7.10 ± 0.62	8.52 ± 1.03	8.74 ± 1.03
20	3.45 ± 0.54	6.49 ± 0.30	10.84 ± 1.92
21	3.81 ± 0.765	13.15 ± 0.44	8.95 ± 1.01
22	4.51 ± 1.30	8.41 ± 3.63	9.61 ± 2.54
23	4.14 ± 0.70	15.10 ± 0.39	27.68 ± 1.91
24	38.50 ± 4.26	59.92 ± 12.70	61.78 ± 2.04
25	6.63 ± 3.37	14.01 ± 1.73	16.88 ± 3.48
26	> 37.5	> 37.5	> 37.5
27	16.08 ± 2.94	16.34 ± 1.40	16.15 ± 0.56

GI_{50} values (**concentration that causes 50% of growth inhibitory effect**) in tumour cells. Cells were **treated for 48 h** and analysed with the **sulforhodamine B assay**.

Results and Discussion

NCI-H460 cells arrest in mitosis, in response to most potent compounds treatment

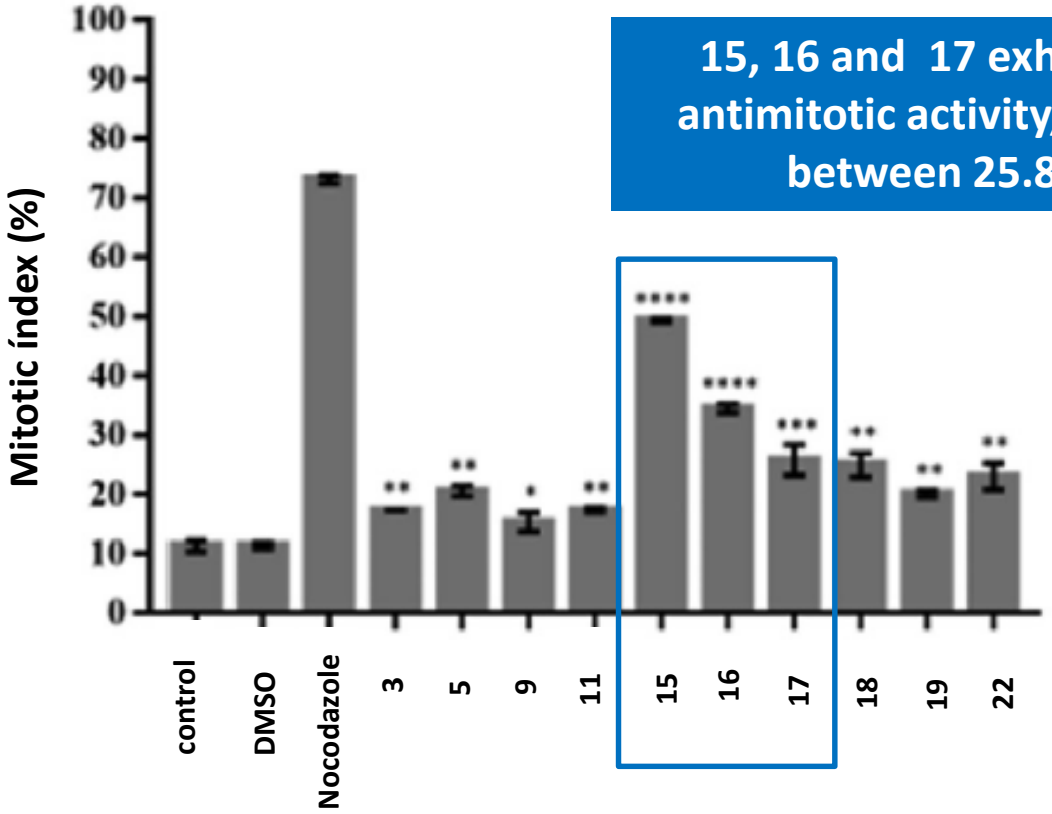


Figure 1. Mitotic Index graph showing accumulation of mitotic cells after 15 h of compound treatment with all selected compounds. Statistical significance of samples treated with the compounds when compared with control (*P < 0.05; **P < 0.01; ***P < 0,001; ****P < 0.0001). Data represent mean±SD of three independent experiments.

Results and Discussion

NCI-H460 cells arrest in mitosis, in response to 15, 16 and 17 treatment

15 h

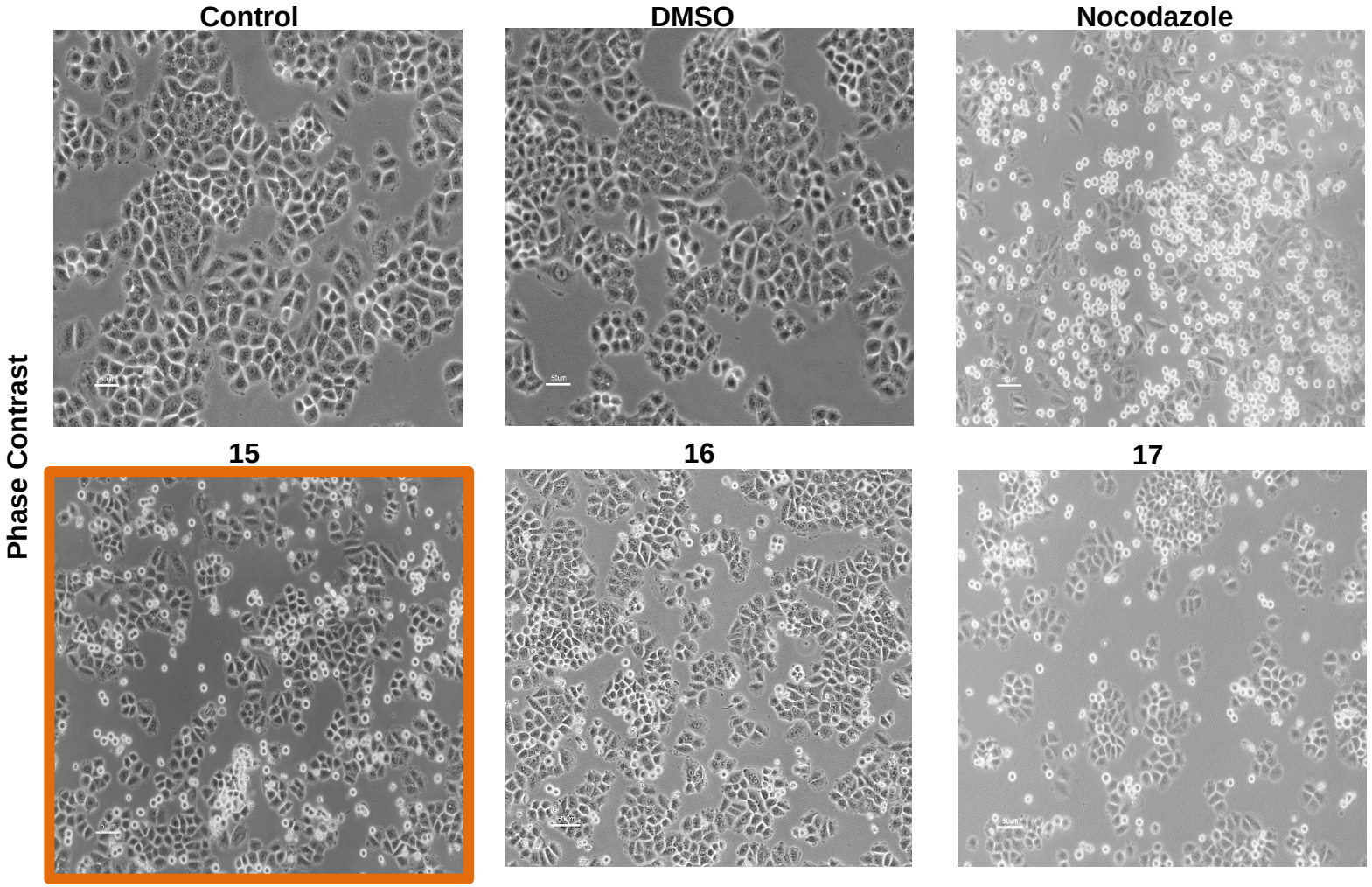


Figure 2. Treatment with **15**, **16** and **17** arrests NCI-H460 cells in mitosis. Phase contrast microscopy images showing an accumulation of rounded-mitotic cells (Bar= 20 µm).

Results and Discussion

NCI-H460 cells arrest in mitosis, in response to 15, 16 and 17 treatment

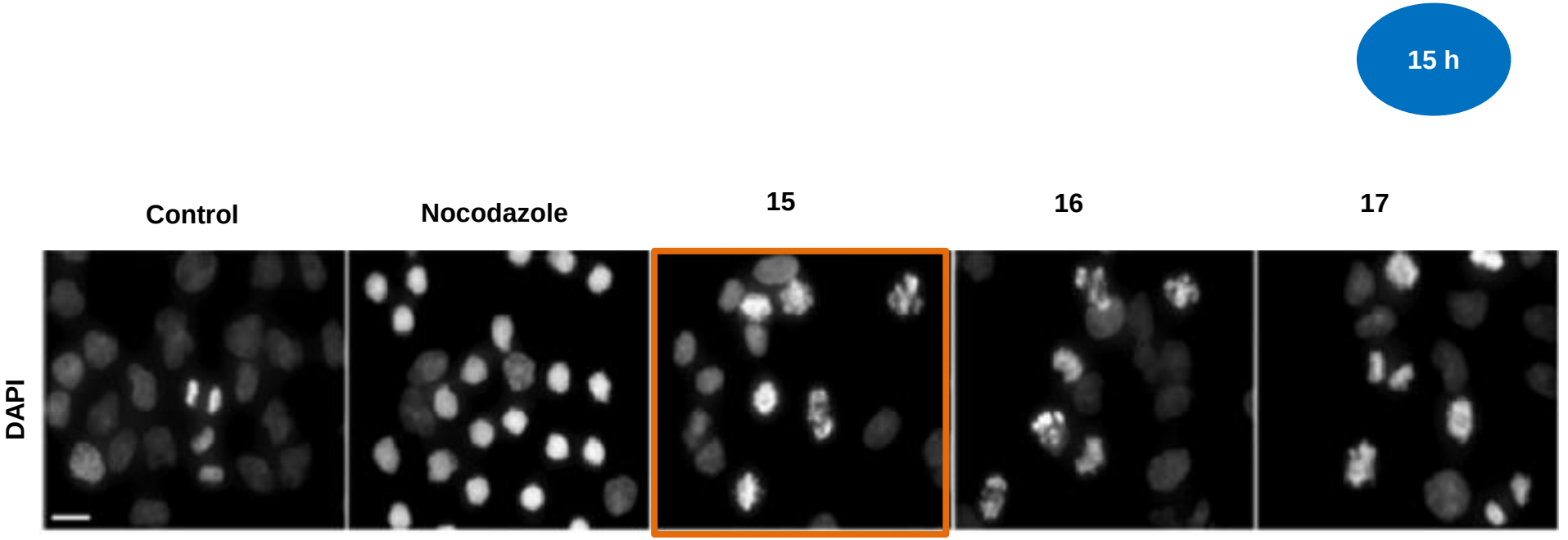
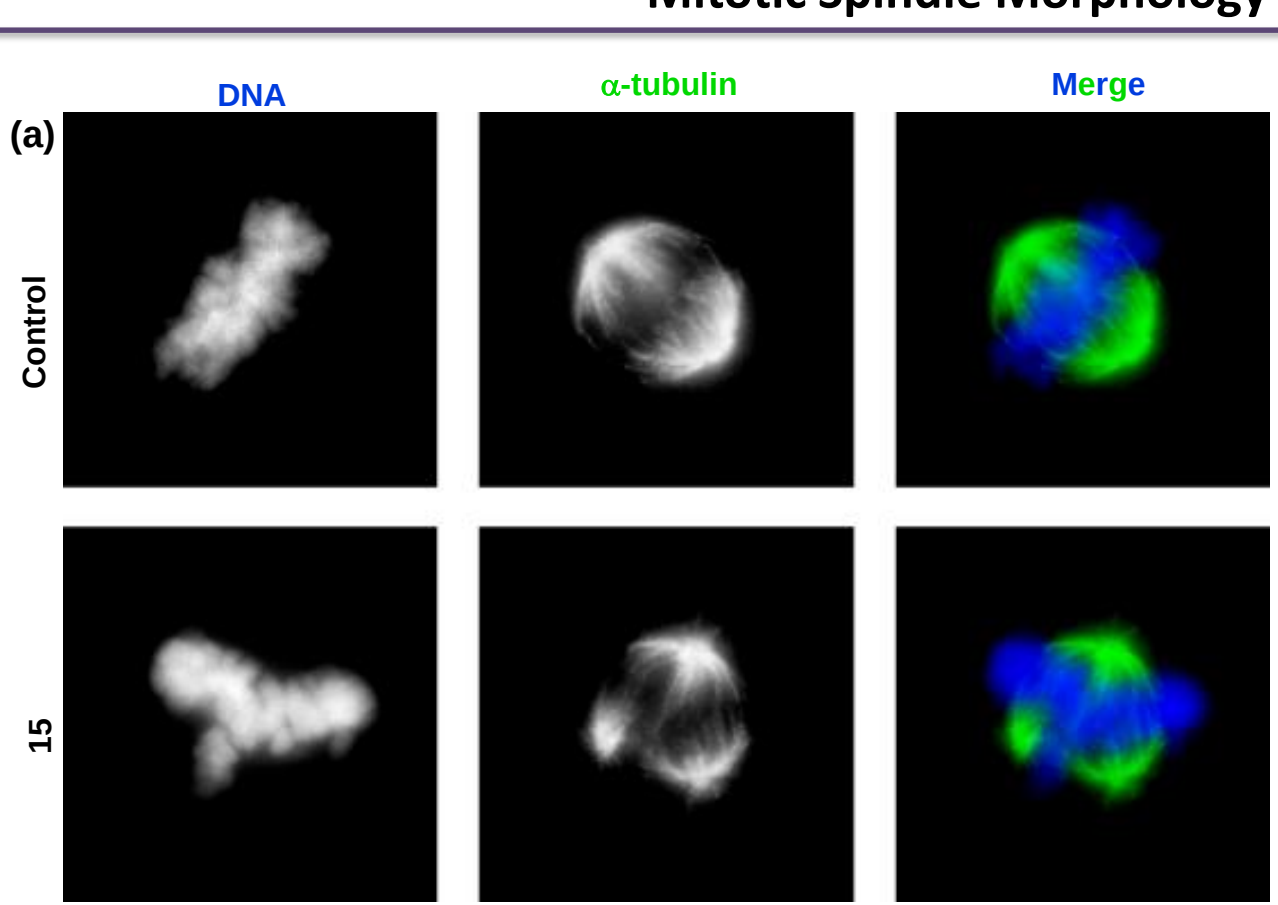


Figure 3. 15, **16** and **17** treatment arrests NCI-H460 cells in mitosis, as shown with DAPI staining of DNA (Bar=5 µm).

Results and Discussion

Mitotic Spindle Morphology



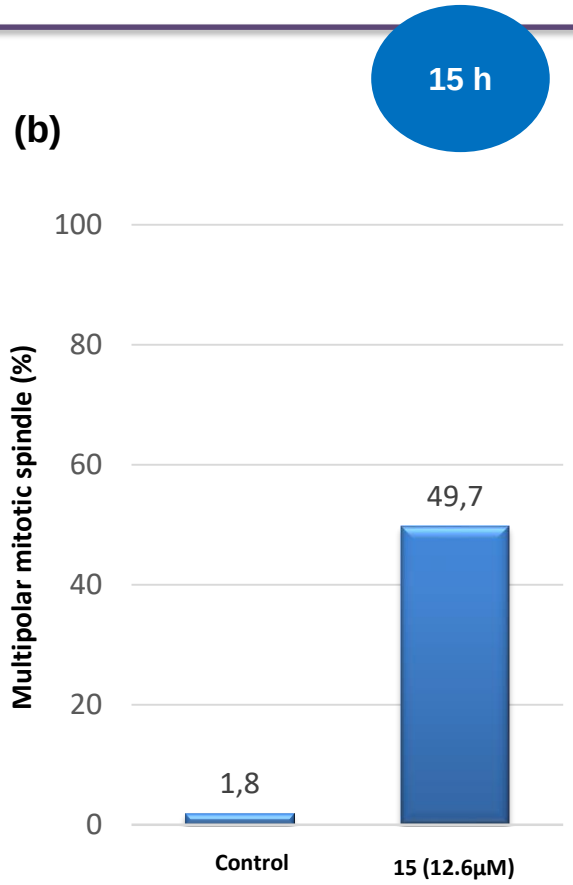


Figure 4. (**a**) **15** treatment affects mitotic spindle morphology. Immunofluorescence staining with anti-α-tubulin antibody. DNA was stained with DAPI (blue). Bar = 5 µm. (**b**) Multipolar mitotic spindle graph showing the percentage of multipolar mitotic spindle in mitotic cells, by 15 hours treatment with 15. Statistical significance of samples with **15** when compared with control (*P<0.05). Data represent mean±SD of three independent experiments.

The same result was obtained for **16** and **17** treatment.

Compound 15 combined treatment with Paclitaxel (Tx)

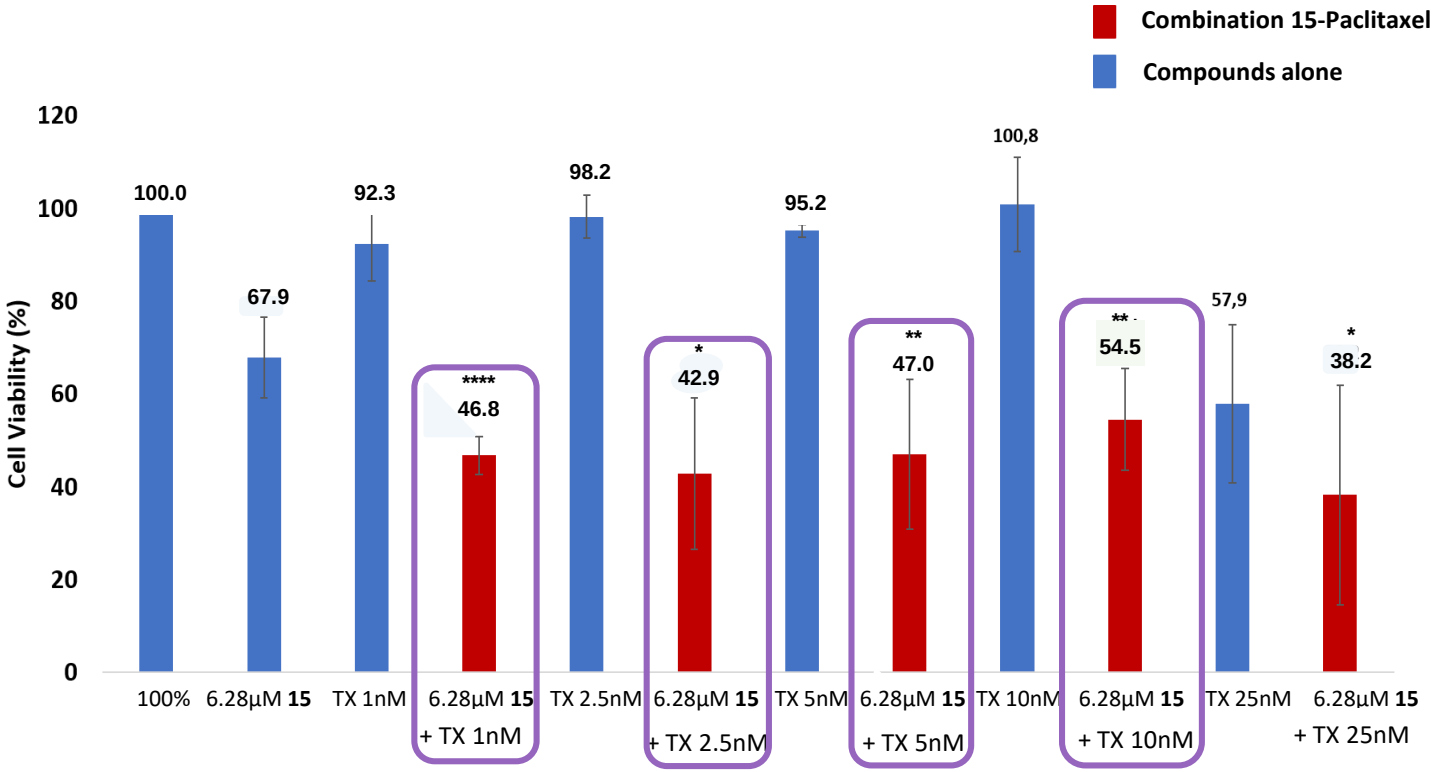
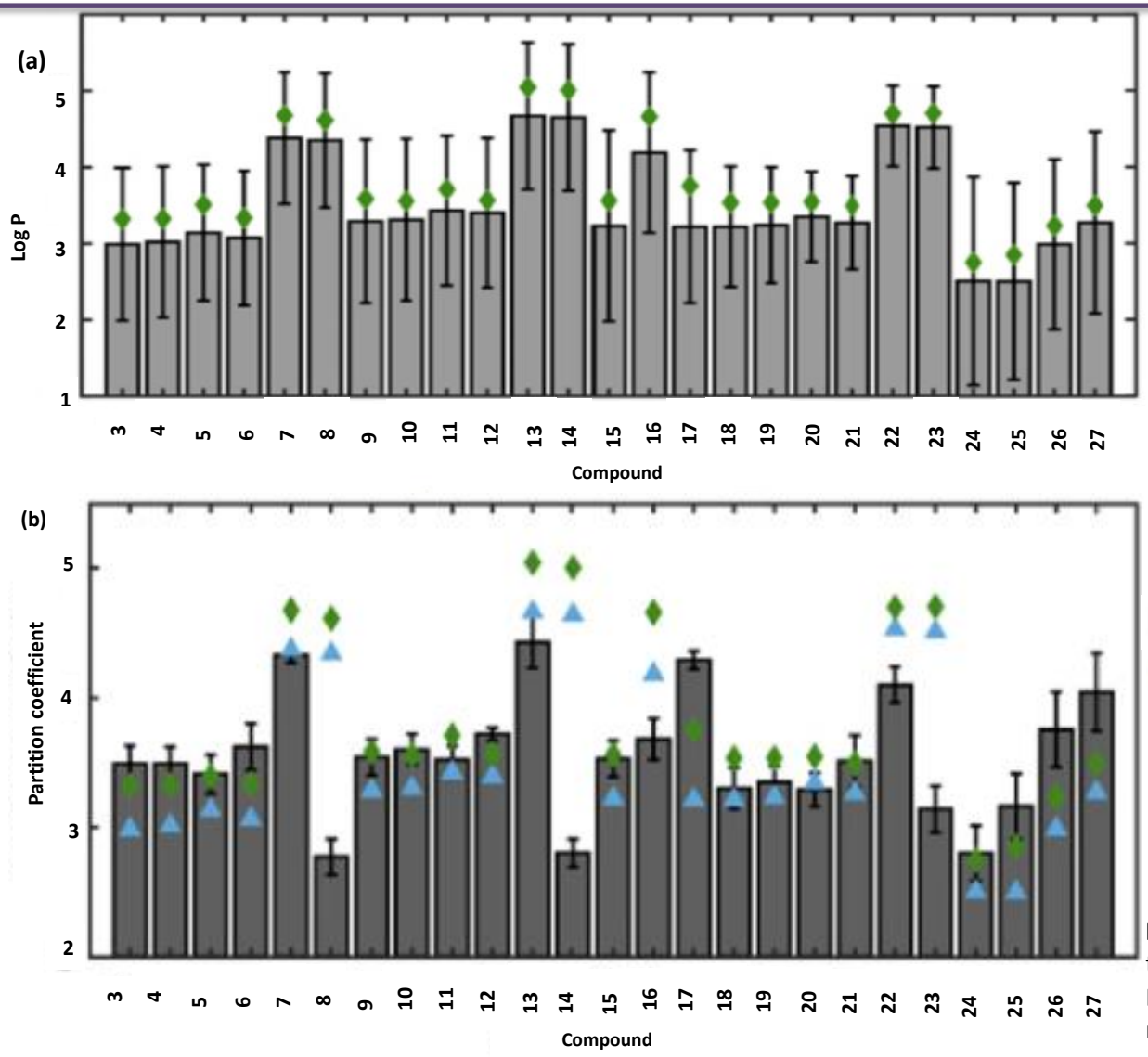


Figure 5. The concentrations of Tx used were from 1 nM to 25 nM as indicated. As control were considered untreated cells. The concentration of Tx at 25 and 2.5 nM with 6.28 µM of **15** presented statistical significance (*p<0.05), Tx at 10 and 5 nM with 6.28 µM of **15** was significant (**p<0.005). Tx at 1 nM combinated with 6.28 µM of **15** had statistical significance (****p<0.0001). Data are means ± SD from at least three independent experiments.

Prediction vs determination of Log P values



Majory of chalcone derivatives:

Predicted log P < determined log Kp

Di-*ortho* chloro-substituted (8, 14 and 23):

Determined log Kp << predicted log P

Figure 6. (a) Mean, median and standard deviation of the Log P predicted by different in silico methods.(b) Experimentally obtained Log Kp values, and mean and median of predicted log P for the studied chalcones.

Results and Discussion

Relationship between Lipophilicity and Antiproliferative activity

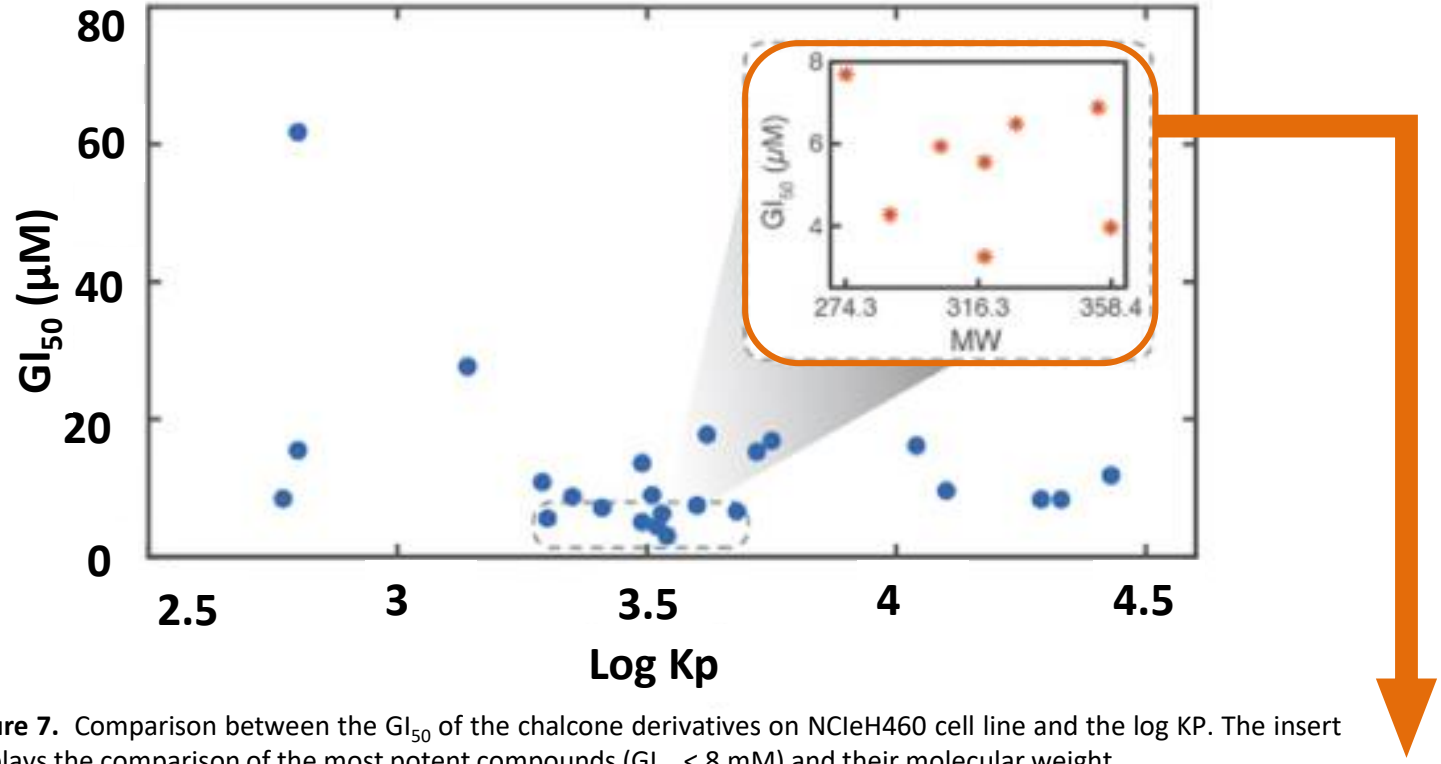


Figure 7. Comparison between the GI_{50} of the chalcone derivatives on NCIeH460 cell line and the log KP. The insert displays the comparison of the most potent compounds (GI_{50} < 8 mM) and their molecular weight.

None compounds showing a GI_{50} < 10 µM → **log Kp below 3**

Most potent compounds (GI_{50} < 8 µM) → **3.30 < lop Kp < 3.68**

Not share the same chemical features (MW)

Conclusions

Synthesis

- 25 chalcone derivatives were synthetized
- **7**, **9**, **10**, **13-17** and **24-27** were described for the first time

Biological Activity

- Chalcones **3**, **5**, **9**, **11**, **15-19**, and **22** demonstrated a potent antiproliferative activity
- Compounds **15-17** emerged as potent antimitotic agents by interfering with mitotic spindle assembly
- Chalcone **15** sensitizes human tumor cells to death by low doses of paclitaxel

Lipophilicity evaluation

- Most potent compounds (GI_{50} < 8 µM) have lop Kp values between 3.30 and 3.60
- Similar lipophilicity do nor share same chemical proprieties (MW)

Acknowledgments

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