Integrated strategies for drug design, synthesis and development.

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SAPIENZA
UNIVERSITÀ DI ROMA

"Tu, disperato pilota, frangi ora fra gli scogli la mia barca già stanca e squassata per tante tempeste!
A te accanto, mio amore! Oh schietto farmacista!
Efficace è la tua droga. Con questo bacio io muoio."
W. Shakespeare. Giulietta e Romeo, Atto 5, Scena 3.

Ultima revisione: 5 dicembre 2013

Lezione 27 – 5 dicembre 2013

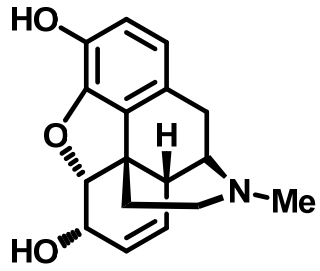
Drug Discovery, Design and Development

The past

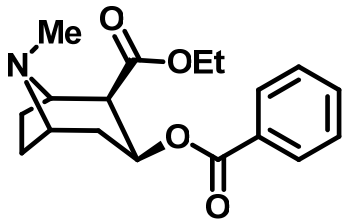
- Before the twentieth century, medicines consisted mainly of herbs and potions and it was not until the mid-nineteenth century that the first serious efforts were made to isolate and purify ***the active principles*** of these remedies.
- The success of these efforts led to the birth of many of the pharmaceutical companies we know today.
- Since then, many naturally occurring drugs have been obtained and their structures determined.

Drug Discovery, Design and Development

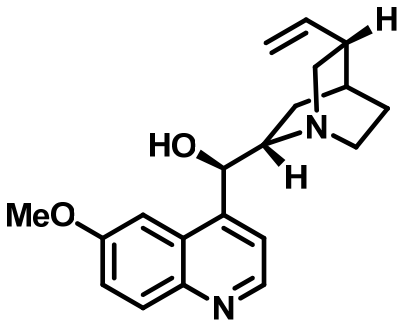
The past



Morphine



Cocaine



Quinine

Drug Discovery, Design and Development

The past

- These natural products sparked off a major synthetic effort where chemists made literally thousands of analogues in an attempt to improve on what nature had provided.
- Much of this work was carried out on a trial and error basis, but the results obtained revealed several general principles behind drug design.

Drug Discovery, Design and Development

The past

- An overall pattern for drug discovery and drug development also evolved, but there was still a high element of trial and error involved in the process.
- The mechanism by which a drug worked at the molecular level was rarely understood, and drug research focused very much on what is known as the ***lead compound***: an active principle isolated from a natural source or a synthetic compound prepared in the laboratory.

Drug Discovery, Design and Development

The present

- In recent years, medicinal chemistry has undergone a revolutionary change.
- Rapid advances in the biological sciences have resulted in a much better understanding of how the body functions at the cellular and the molecular level.
- As a result, most research projects in the pharmaceutical industries or accademia now begin by identifying a suitable target in the body and designing a drug to interact with that target.

Drug Discovery, Design and Development

The present

- An understanding of the structure and function of the target, as well as the mechanism by which it interacts with potential drug is crucial to this approach.
- Generally, we can identify the following stages:
  - ✓ drug discovery;
  - ✓ drug design;
  - ✓ drug development.

Drug Discovery, Design and Development

Drug discovery

- Choose a disease!
- Choose a drug target.
- Identify a bioassay.
- Find a ‘hit compound’.
- Isolate and purify the hit compound(s) if necessary.

Drug Discovery, Design and Development

Drug design

- Identify structure-activity relationships (SARs).
- Identify the pharmacophore.
- Improve target interactions (pharmacodynamics).
- Improve pharmacokinetic properties.

Drug Discovery, Design and Development

Drug development

- Patent the drug.
- Carry out preclinical trials (drug metabolism, toxicology, formulation and stability tests, pharmacology studies, etc).
- Design a manufacturing process (chemical and process development).
- Carry out clinical trials.
- Register and market the drug.
- Make money!

Drug Discovery, Design and Development

The present

- Many of these stage run concurrently and are dependent on each other.
- For example, preclinical trials are usually carried out in parallel with the development of a manufacturing process.
- Even so, the discovery, design and development of a new drug can take 15 years or more, involve the synthesis of over 10000 compounds, and cost in the region of 800 million US dollars or 560 million euros.

Drug Design and Synthesis Center

Research team

- ***Permanent staff***

  Prof Romano Silvestri and Dr Giuseppe La Regina

- ***Post-doc***

  Dr Antonio Coluccia, Dr Sveva Pelliccia

- ***PhD students***

  Dr Valeria Famiglini, Dr Sara Passacantilli

- ***Graduated and graduating students***

  Tullio Viti, Matteo Creta, Enrica Loi, Valeria D'Amico, Marianna Mele, Marina Rocco, Federica Carpinella

Drug Design and Synthesis Center

Research team

Drug Design and Synthesis Center

Main facilities

- CEM Discover SP focused microwave reactor
- Interchim Spot II Flash fully automated flash chromatography system
- Dionex UltiMate 3000 Thermo Scientific HPLC system
- 6 Buchi Rotavapors (R-210 and R-II) equipped with Buchi vacuum controllers (V-850 and V-855) and Buchi vacuum (V-700 and V-710, 5 and 2 mbar, respectively) and VacuuBrand high-vacuum (RC 6, $2 \cdot 10^{-3}$ mbar) oil pump.
- Bruker Avance 400 MHz NMR spectrometer
- PerkinElmer ATR-FTIR SpectrumOne spectrometer
- 2 MacPro dual 2.66 GHz Xeon

Choosing a Disease

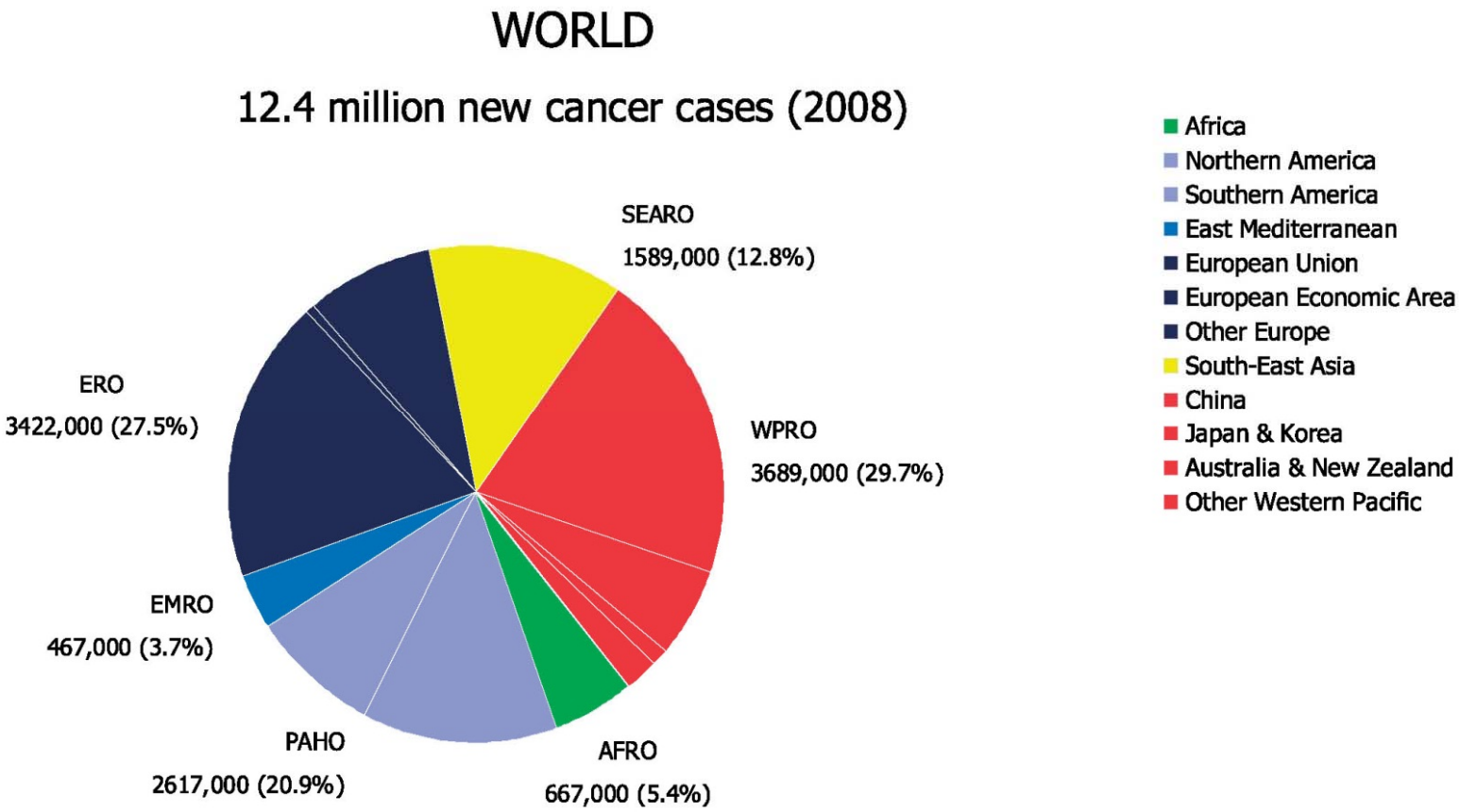
Cancer

- Cancer is projected to become the leading cause of death worldwide in the year 2010.
- Cases of cancer doubled globally between 1975 and 2000, will double again by 2020, and will nearly triple by 2030.
- There were an estimated 12 million new cancer diagnoses and more than 7 million deaths worldwide in 2008. The projected numbers for 2030 are 20 to 26 million new diagnoses and 13 to 17 million deaths.

World Cancer Report **2008**

Choosing a Disease

Cancer

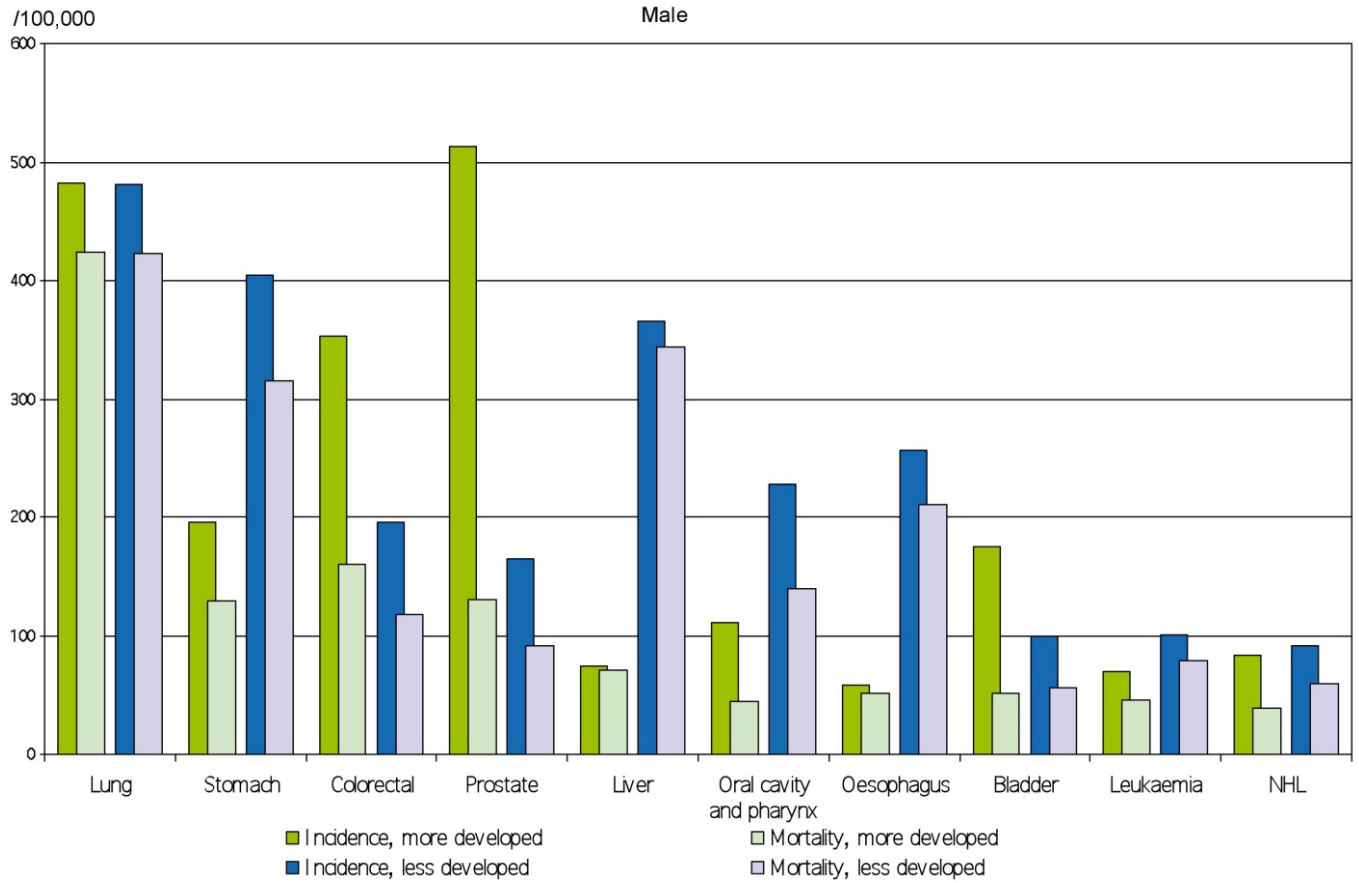


Distribution of global cancer burden by World Health Organization region

World Cancer Report **2008**

Choosing a Disease

Cancer

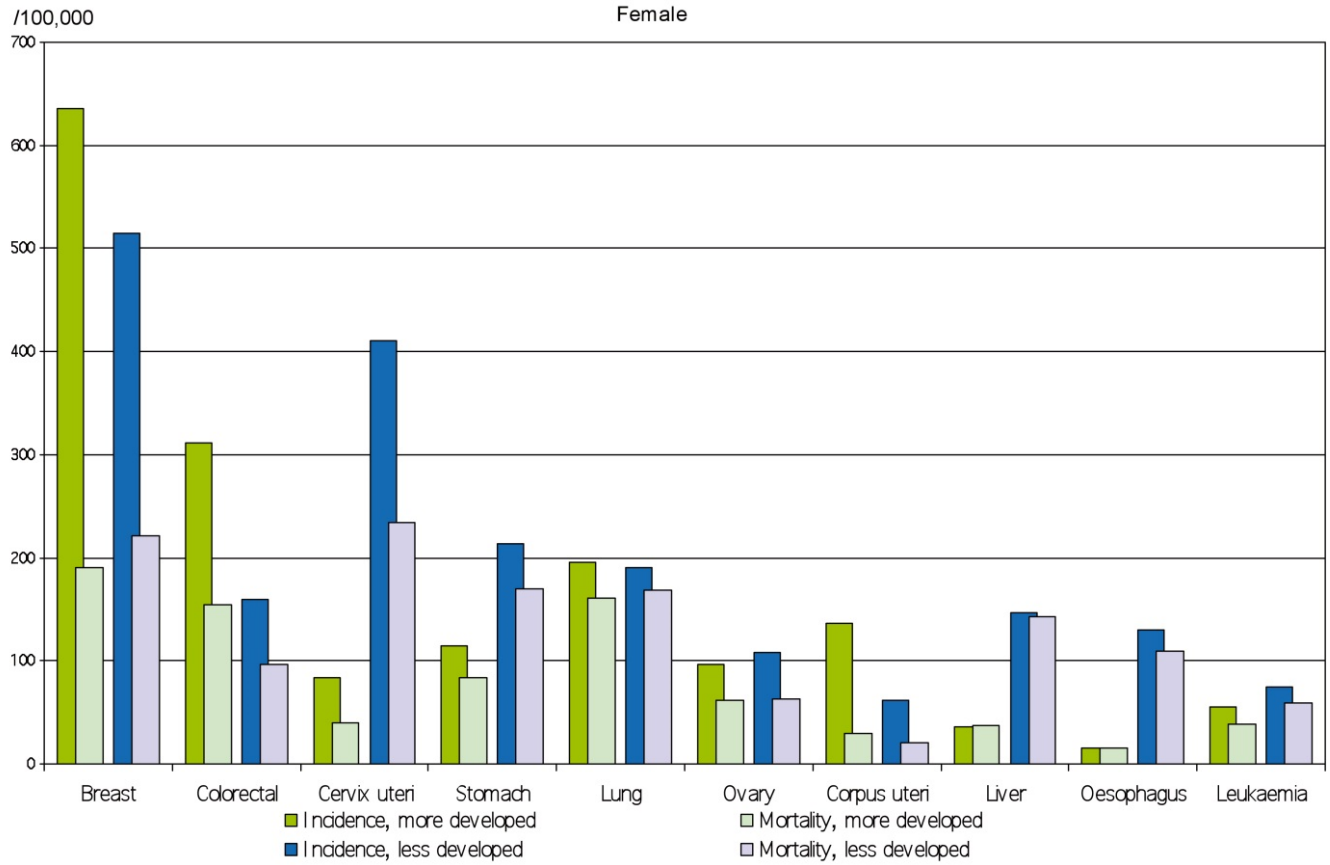


The incidence and mortality of the most common cancers in males in more-developed and less-developed countries.

World Cancer Report **2008**

Choosing a Disease

Cancer

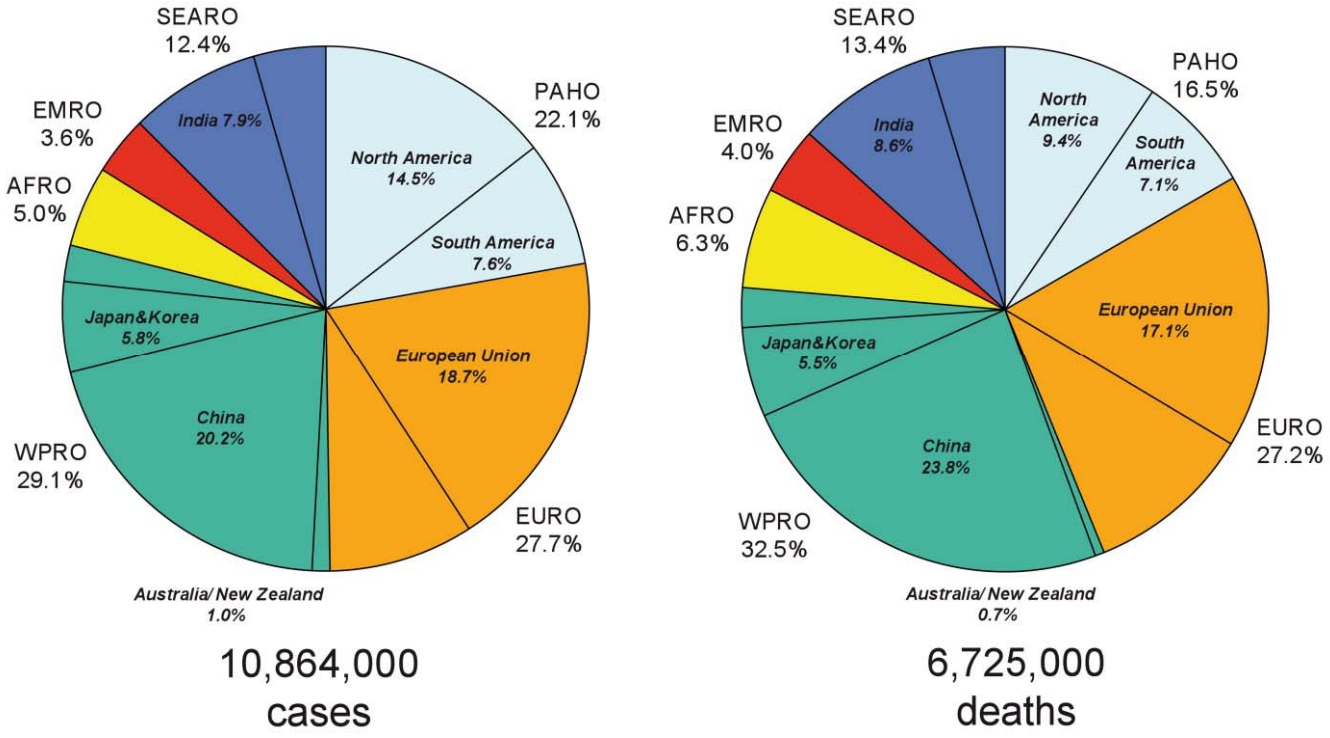


The incidence and mortality of the most common cancers in females in more-developed and less-developed countries.

World Cancer Report **2008**

Choosing a Disease

Cancer



Incidence and mortality in the six WHO world areas [AFRO: Africa; EMRO: East Mediterranean; EURO: Europe; PAHO: PanAmerican; SEARO: South-East Asia; WPRO: Western Pacific].

World Cancer Report **2008**

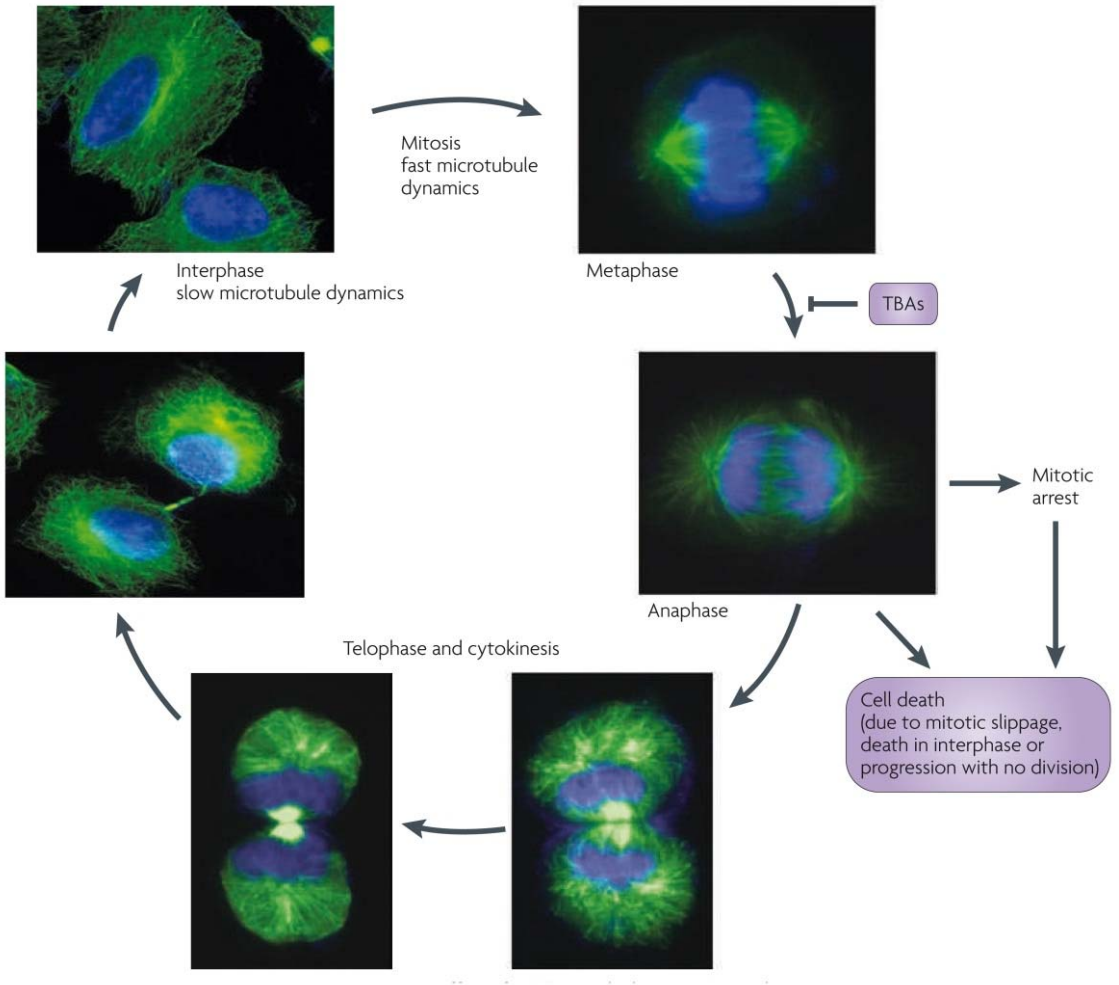
Choosing a Drug Target

Tubulin and microtubules

- Microtubules are involved in a wide range of cellular functions and are critical to the life cycle of the cell.
- Composed of linear rows of alternating α- and β-tubulin, microtubules are highly dynamic and rapidly assemble and disassemble to meet the cell's needs.
- Since inhibition of tubulin polymerization or blockage of microtubule disassembly increases the number of cells in metaphase arrest, microtubules are attractive molecular targets for anticancer therapeutics.

Choosing a Drug Target

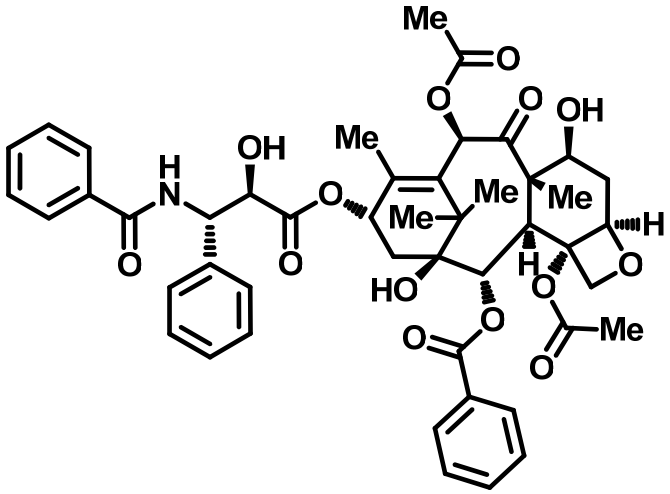
Tubulin and microtubules



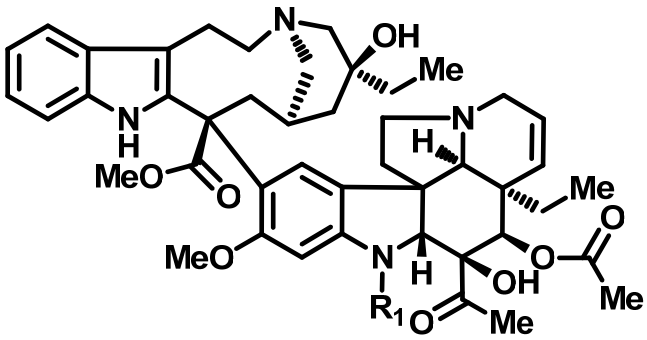
Kavallaris, M. *Nat. Rev. Cancer* **2010**, *10*, 1-11.

Choosing a Drug Target

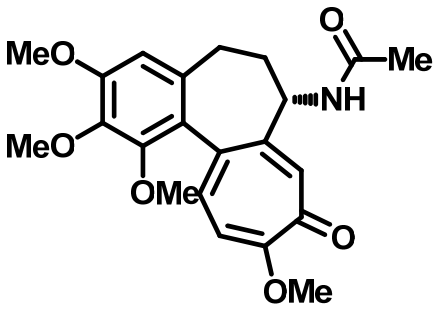
Tubulin inhibitors



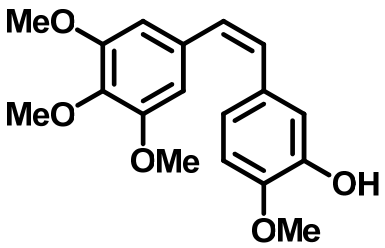
Taxol



R_1 = CHO, Vincristine
R_1 = Me, Vinblastine



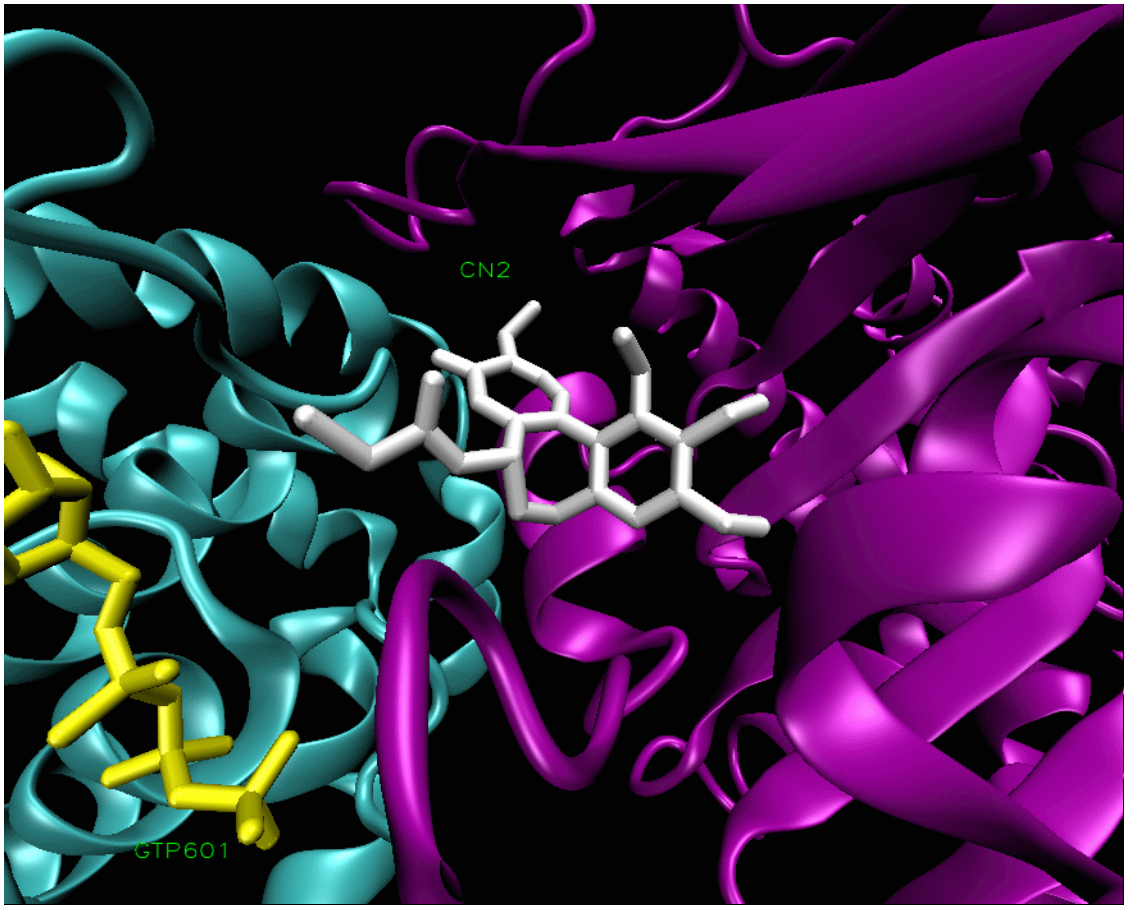
Colchicine



Combretastatin A4

Choosing a Drug Target

Tubulin binding sites



α,β-Tubulin/DAMA-colchicine complex

Lowe, J. *et al. Nature* **2004**, *428*, 198-202 (PDB code: 1SA0).

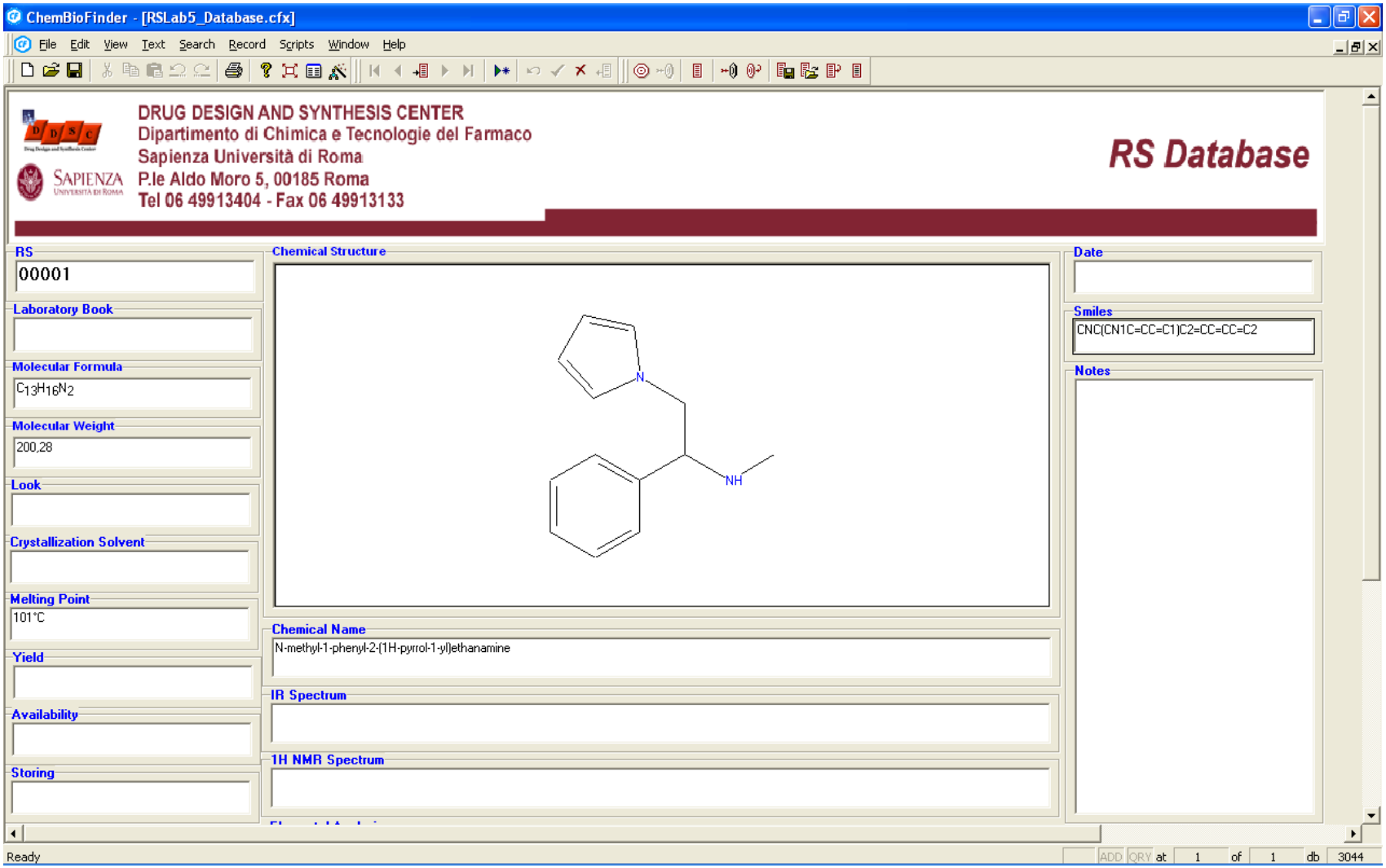
Choosing a Drug Target

New anti-tubulin agents

- Paclitaxel and vinca alkaloids are clinically important chemotherapeutic drugs, and are widely used for the treatment of a variety of tumours.
- Restrictions due to toxicity, drug resistance, complex formulations and limited bioavailability highlight the need for novel tubulin inhibitors.
- Furthermore, while drugs that act on the vinca and taxane sites have well-established roles in the treatment of human cancers, the therapeutic potential of the colchicine site in cancer treatment has yet to be realized.

Finding a hit compound

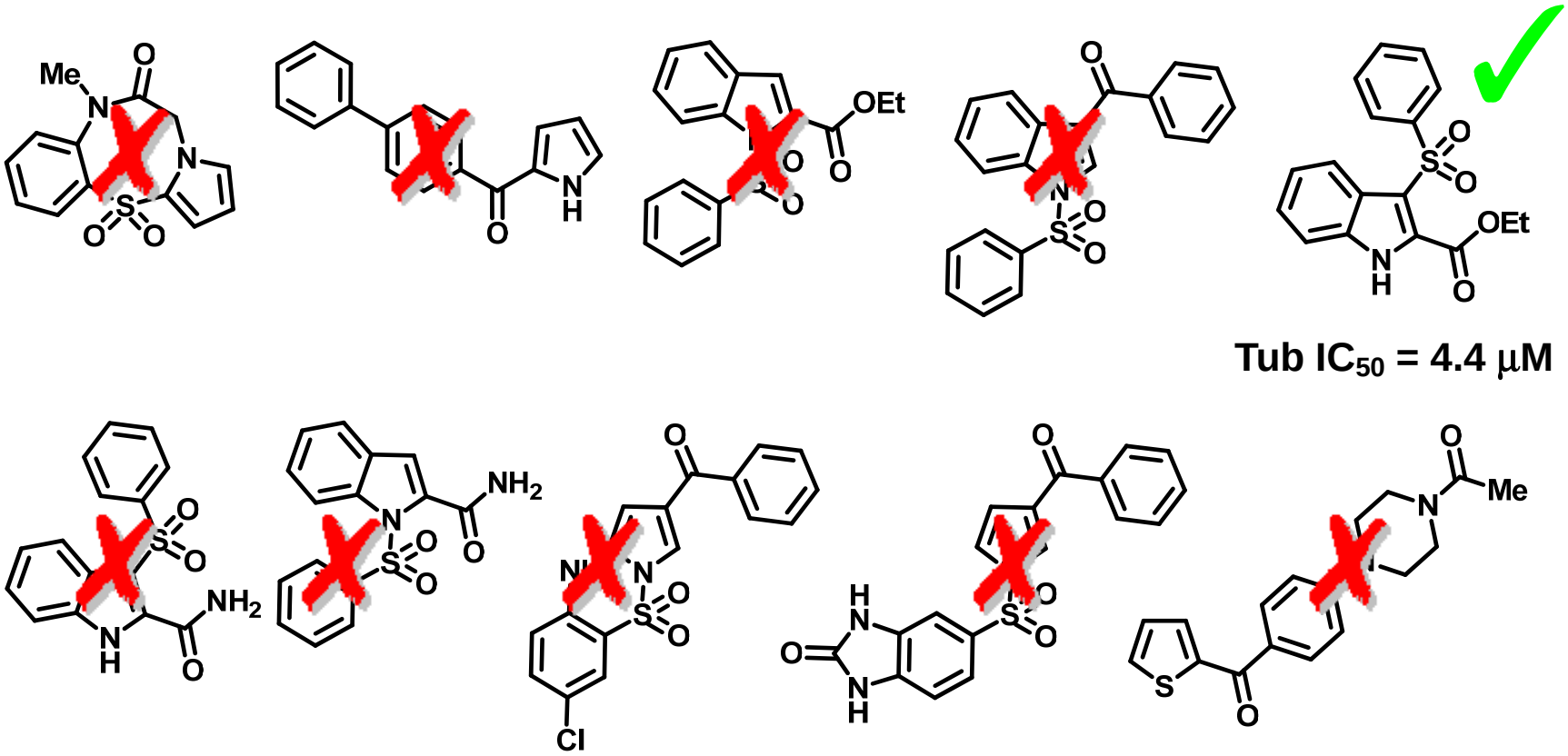
Screening at National Cancer Institute



Dr G. La Regina. *Integrated strategies for drug design, synthesis and development.*

Finding a hit compound

Screening at National Cancer Institute



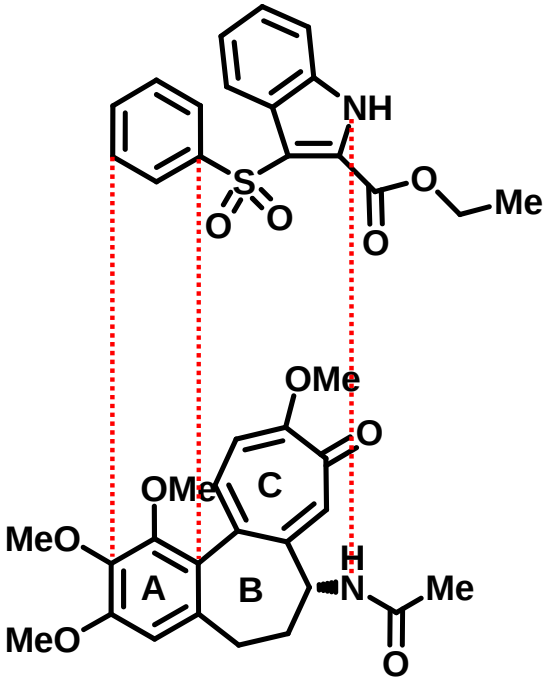
Tub IC_{50} = 4.4 μM

> 50 compounds were biologically evaluated

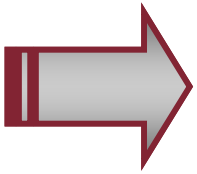
Arylthioindoles: 1st Series

Rational

Hit compound

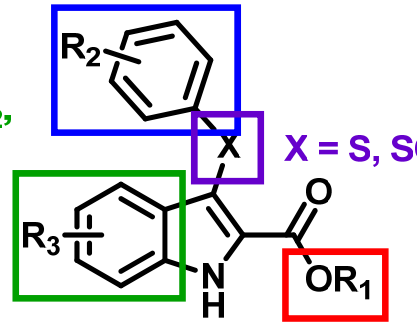


Colchicine



R_2 = H, 2-OMe, 3-OMe, 4-OMe, 3,5-$(OMe)_2$, 3,4,5-$(OMe)_3$, 3,5-$(Me)_2$

R_3 = H, 4-OMe, 5-Cl, 5-NO_2, 5-OMe, 6-OMe, 5,6-$(OMe)_2$



X = S, SO_2

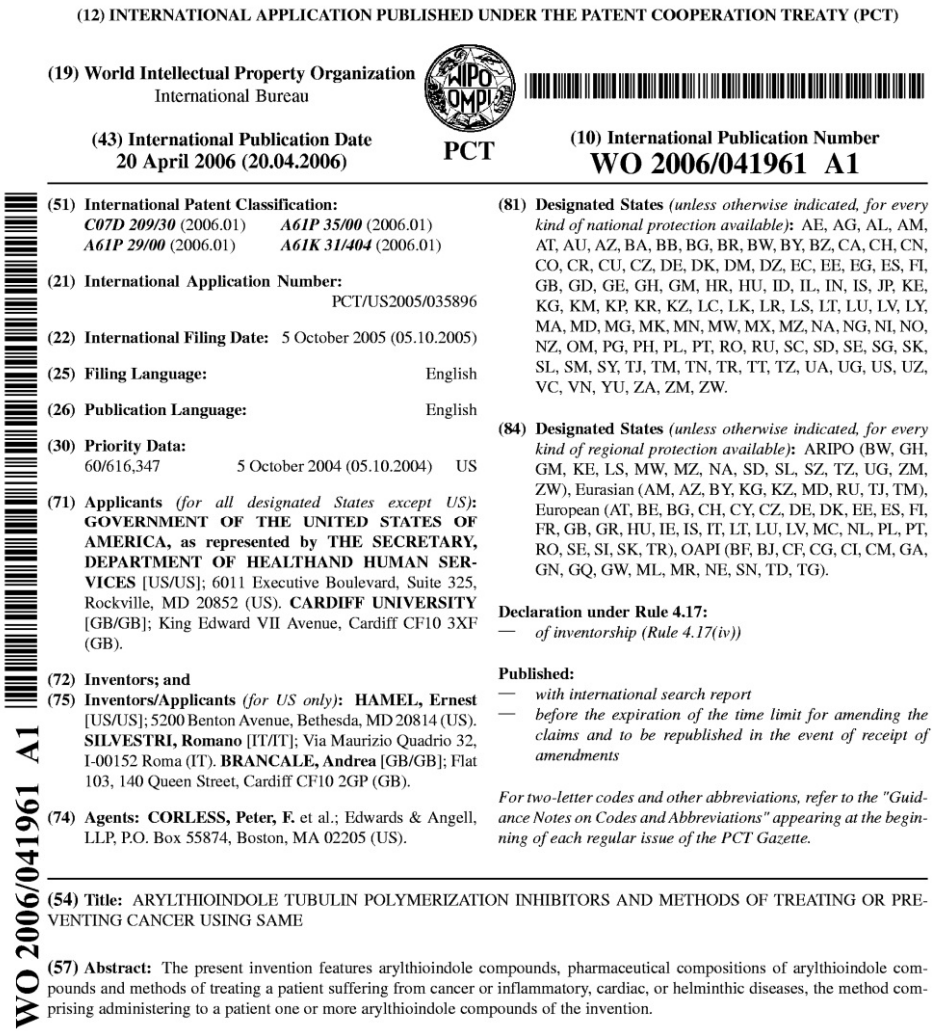
37 new ATIs

R_1 = Me, Et, *n*-Pr, *i*-Pr, *n*-Bu, *s*-Bu, *t*-Bu, *i*-Pe, Bn

J. Med. Chem. **2004**, *47*, 6120-6123. *PCT Int. Appl.* **2006**, WO041961. *J. Med. Chem.* **2006**, *49*, 947-954.

Arylthioindoles: 1st Series

Rational



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A61P 29/00 (2006.01) *A61K 31/404* (2006.01)

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(74) Agents: **CORLESS, Peter, F.** et al.; Edwards & Angell, LLP, P.O. Box 55874, Boston, MA 02205 (US).

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Declaration under Rule 4.17:
— *of inventorship (Rule 4.17(iv))*

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

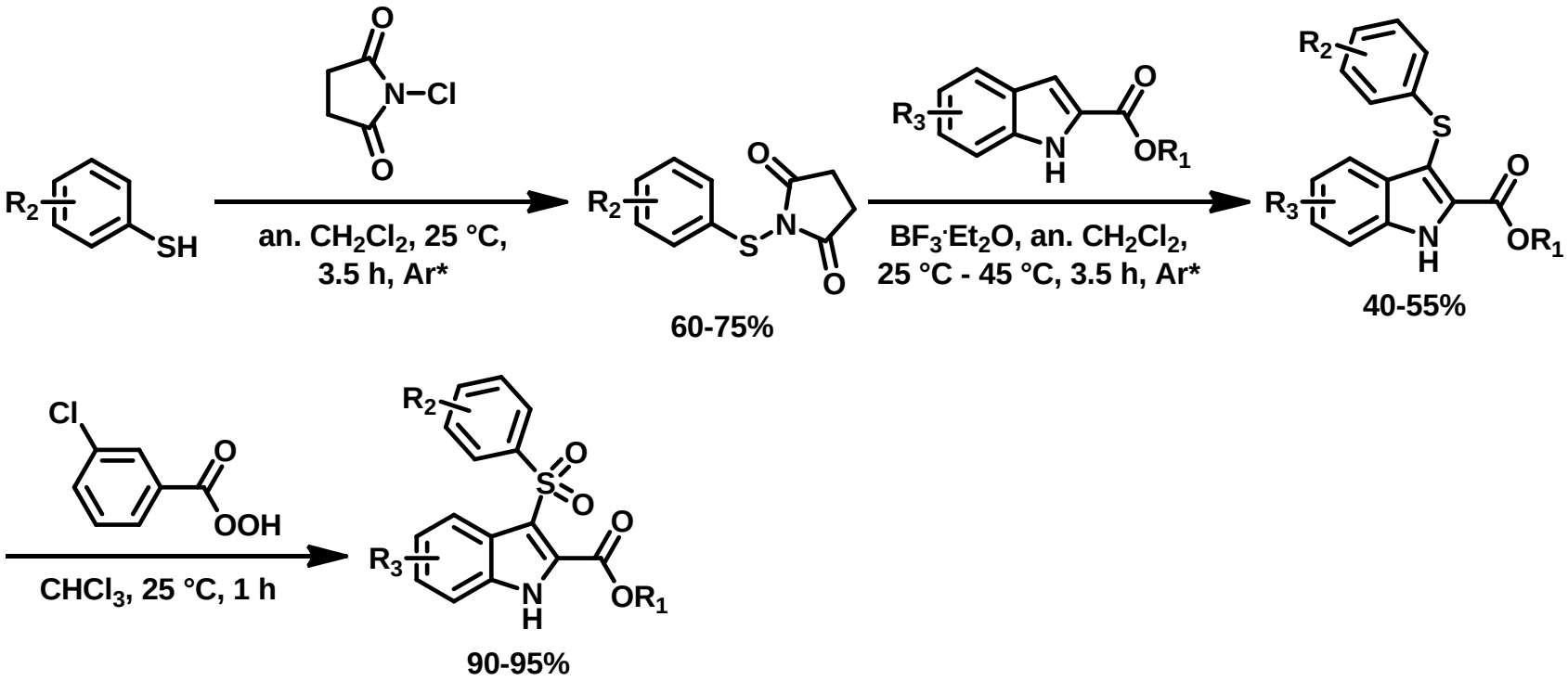
For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: ARYLTHIOINDOLE TUBULIN POLYMERIZATION INHIBITORS AND METHODS OF TREATING OR PREVENTING CANCER USING SAME

(57) Abstract: The present invention features arylthioindole compounds, pharmaceutical compositions of arylthioindole compounds and methods of treating a patient suffering from cancer or inflammatory, cardiac, or helminthic diseases, the method comprising administering to a patient one or more arylthioindole compounds of the invention.

Arylthioindoles: 1st Series

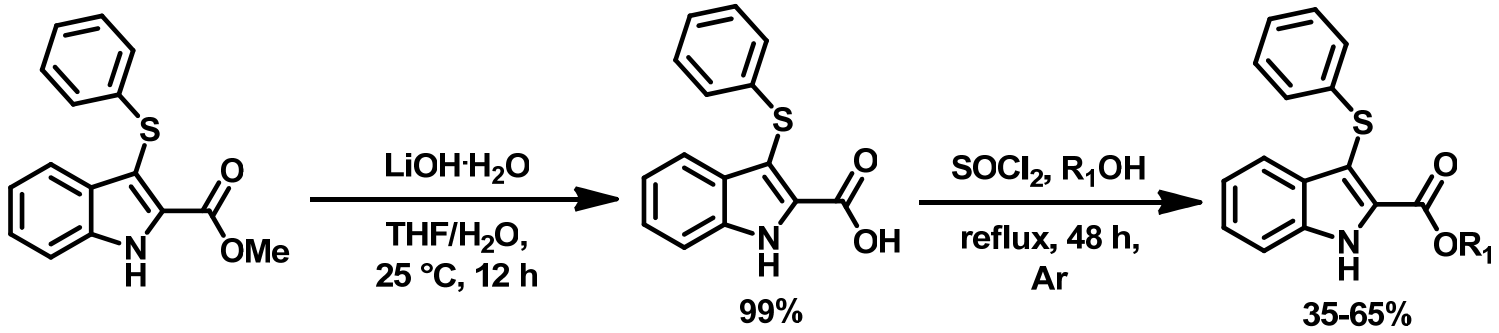
Synthesis



*R_1 = Me, Et; R_2 = 2-OMe, 3-OMe, 4-OMe, 3,5-Me_2.

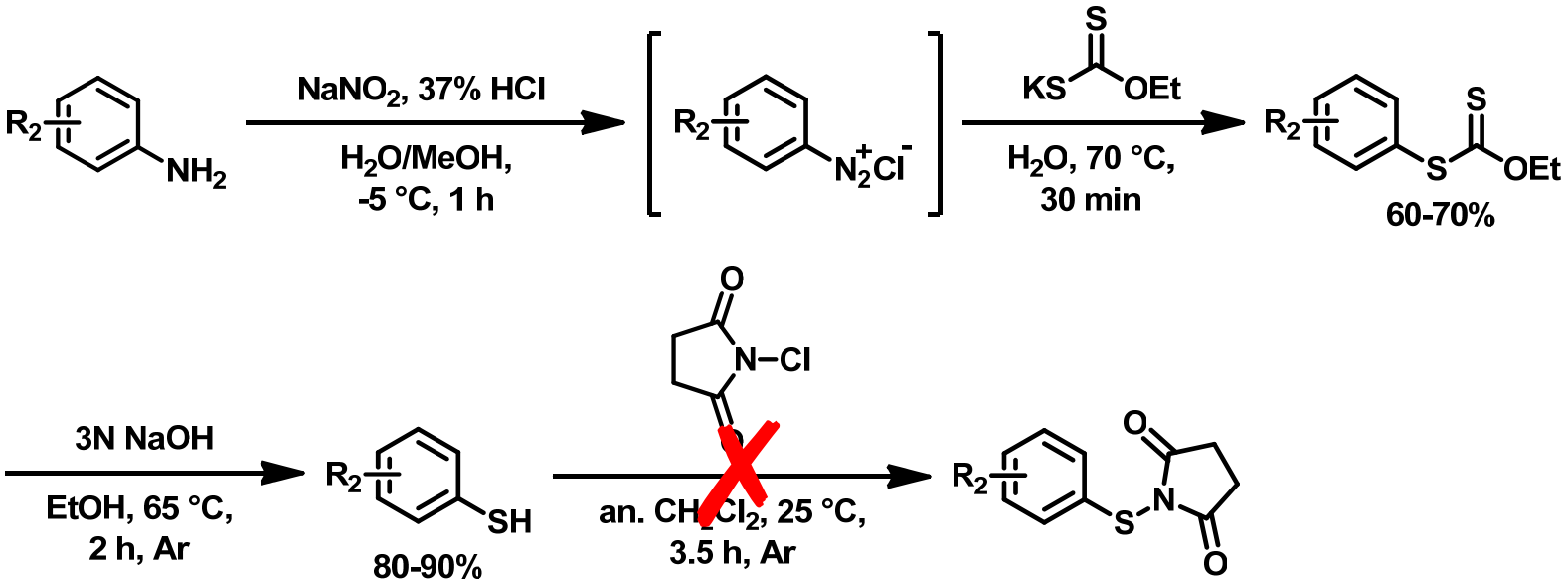
Arylthioindoles: 1st Series

Synthesis



Arylthioindoles: 1st Series

Synthesis

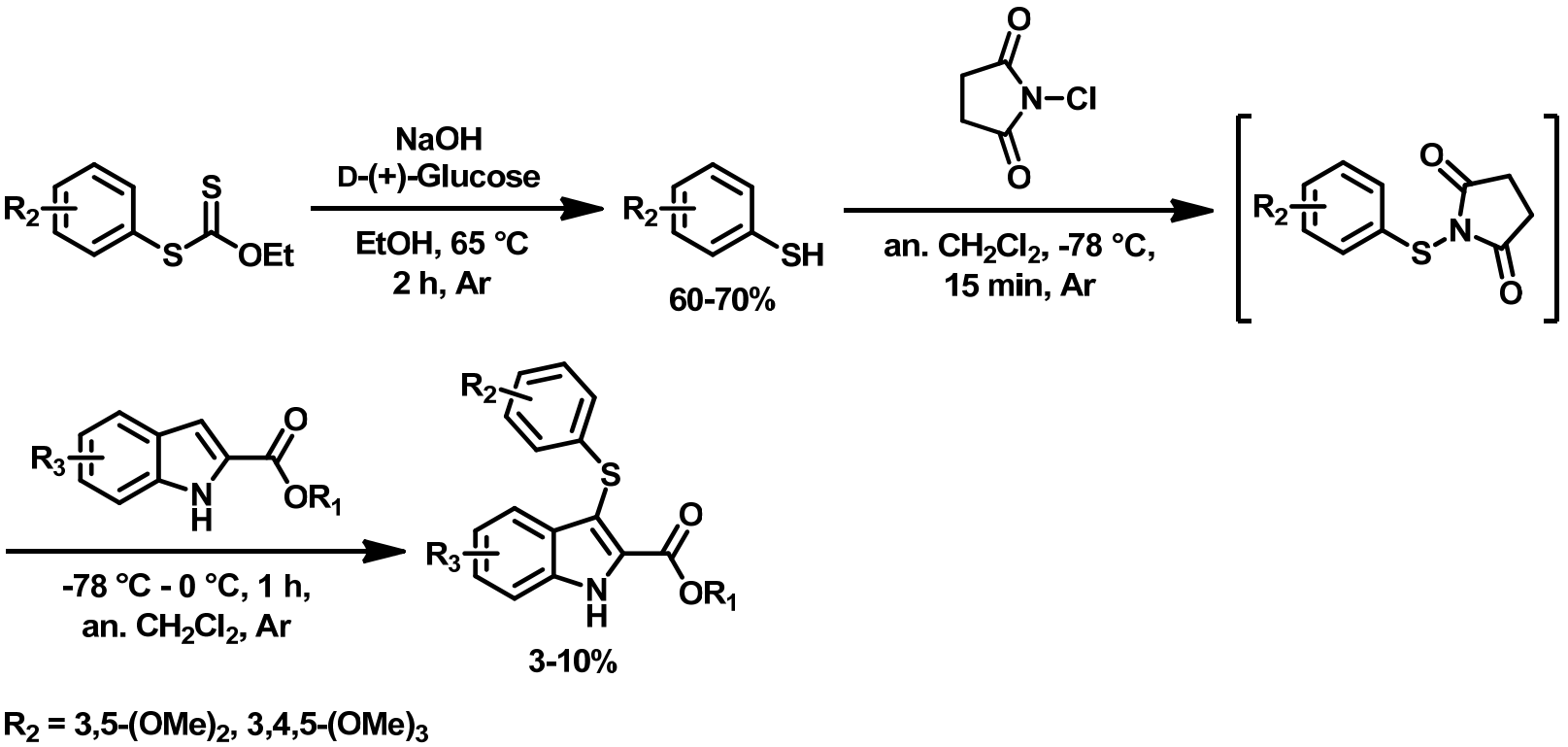


R_2 = 3,5-$(OMe)_2$, 3,4,5-$(OMe)_3$

Offer, J. *et al. J. Am. Chem. Soc.* **2002**, *124*, 4642-4646.

Arylthioindoles: 1st Series

Synthesis

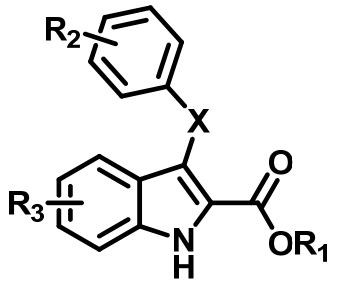


R_2 = 3,5-$(OMe)_2$, 3,4,5-$(OMe)_3$

Schlosser, K. M. *et al. Org. Lett.* **2004**, *6*, 819-821.

Arylthioindoles: 1st Series

Biological activity



1-13

Cpd	R_1	R_2	R_3	X	Tubulin[a] IC_{50} ± SD (μM)	MCF-7[b] IC_{50} ± SD (nM)	Colchicine binding[c] (% ± SD)	SCLC[d] IC_{50} ± SD (nM)
1	Me	H	H	S	8.3 ± 0.6	>2500	21 ± 7	-
2	Et	H	H	S	4.4 ± 0.3	>1250	19 ± 7	-
3	Et	2-OMe	5-OMe	S	16 ± 0.5	350 ± 60	-	2200 ± 200
4	Et	3-OMe	5-OMe	S	3.1 ± 0.2	280 ± 100	39 ± 3	584 ± 40
5	Et	4-OMe	5-OMe	S	>40	>2500	-	>10000
6	Et	3,4,5-$(OMe)_3$	H	S	2.9 ± 0.2	40 ± 2	51 ± 3	84 ± 5
7	Me	3,4,5-$(OMe)_3$	5-Cl	S	2.5 ± 0.3	42 ± 10	57 ± 2	216 ± 17
8	Et	3,4,5-$(OMe)_3$	5-Cl	S	2.2 ± 0.2	110 ± 20	53 ± 6	93 ± 10
9	Me	3,4,5-$(OMe)_3$	5-Cl	SO_2	>40	>2.5	1.6 ± 2	-
10	Me	3,4,5-$(OMe)_3$	5-OMe	S	2.0 ± 0.2	13 ± 3	90 ± 1	47 ± 2
11	Et	3,4,5-$(OMe)_3$	5-OMe	S	2.4 ± 0.2	46 ± 3	71 ± 2	-
12	Me	3,4,5-$(OMe)_3$	5,6-$(OMe)_2$	S	>40	1600 ± 400	-	-
13	Et	3,4,5-$(OMe)_3$	5,6-$(OMe)_2$	S	22 ± 0.7	1000 ± 200	-	-
Colch[e]					3.2 ± 0.2	13 ± 3	-	-
CSA4[f]					2.2 ± 0.2	17 ± 10	97 ± 0.5	-

[a]Inhibition of tubulin polymerization. [b]Inhibition of growth of MCF-7 human breast carcinoma cells. [c]Inhibition of [^{3}H]colchicine binding. [d]Inhibition of growth of SCLC cells. [e]Colchicine. [f]Combretastatin A4.

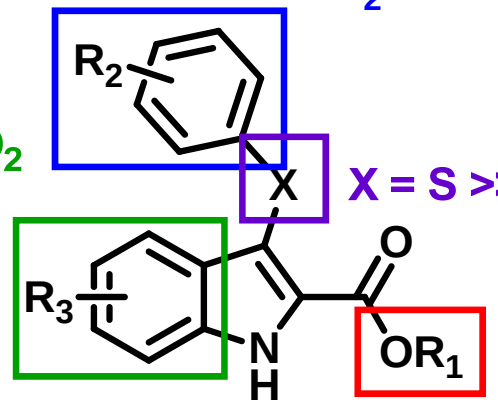
Arylthioindoles: 1st Series

SARs

R_2 = 3,4,5-$(OMe)_3$, ***preferred***
R_2 = 3,5-$(OMe)_2$, 2-OMe, 3-OMe, ***good***
R_2 = 4-OMe, 3,5-Me_2, ***bad***

4-H, 6-H >> 4-OMe, 6-OMe

5-OMe > 5-Cl ≈ 5-H >> 5-NO_2



X = S >> SO_2

R_1 = Small ester (Me, Et), ***preferred***
Carboxylic acid, carboxamide, ***inactive***

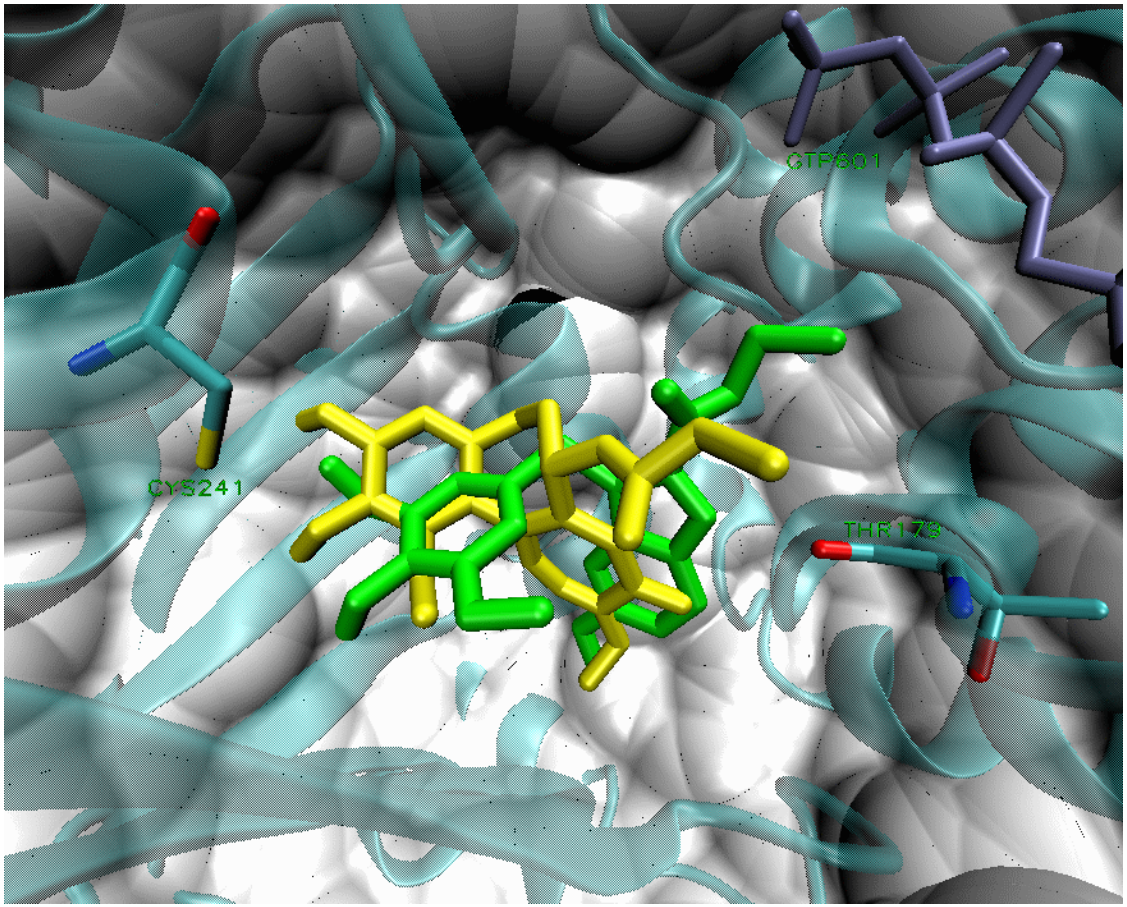
Arylthioindoles: 1st Series

Docking studies

- Semi-flexible docking studies (genetic algorithm global search method) were performed using MOE 2004.03 software package into the colchicine binding site of tubulin (PDB code: 1SA0).
- All the minimizations were performed with MOE until RMSD gradient of 0.001 kcal mol^{-1} $Å^{-1}$ was reached with the MMFF94x force field. The partial charges were automatically calculated.
- The second highest scored pose was selected and the protein/ligand complex was minimized.

Arylthioindoles: 1st Series

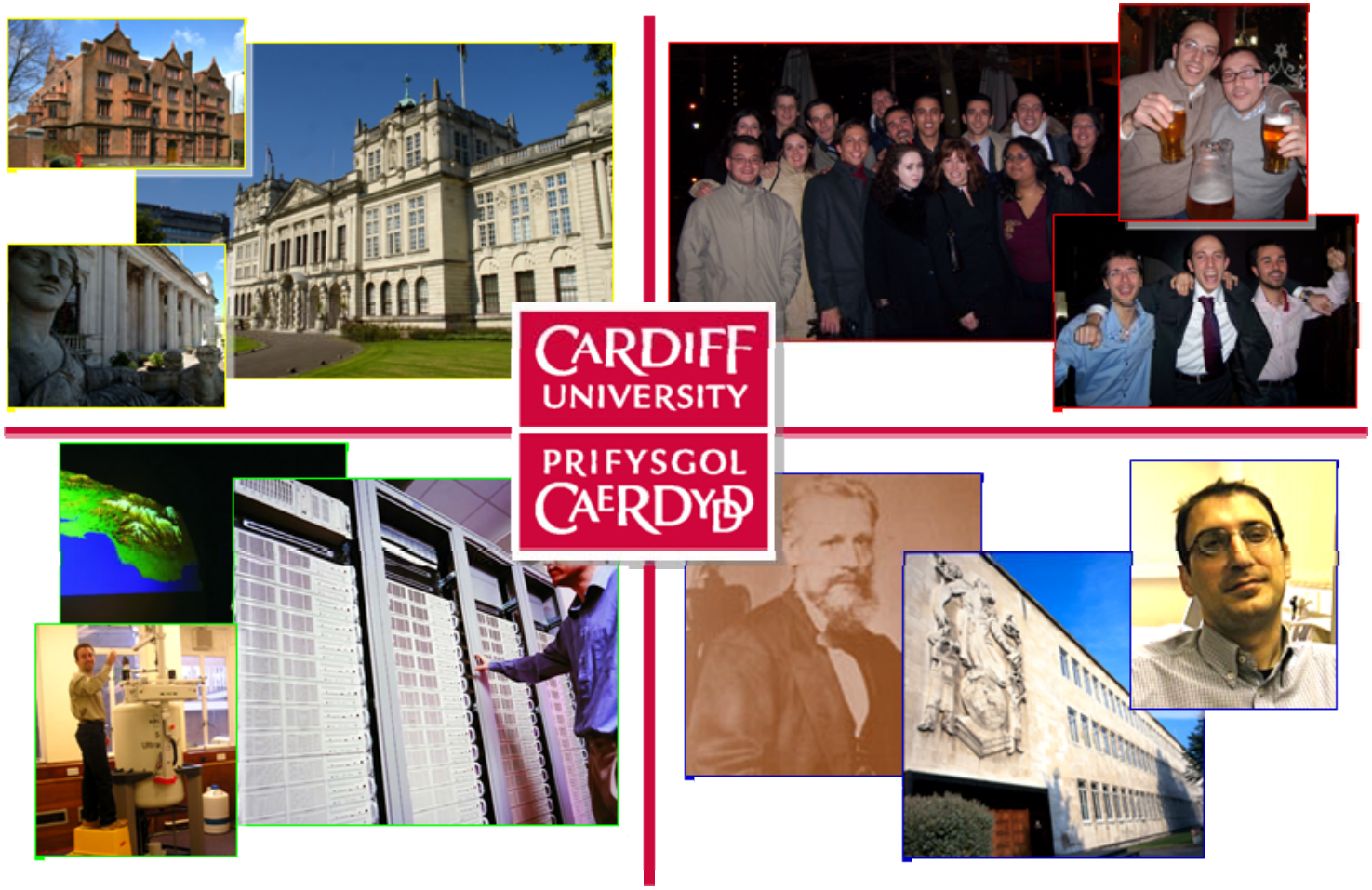
Docking studies



MOE binding mode

Arylthioindoles: 1st Series

Docking studies



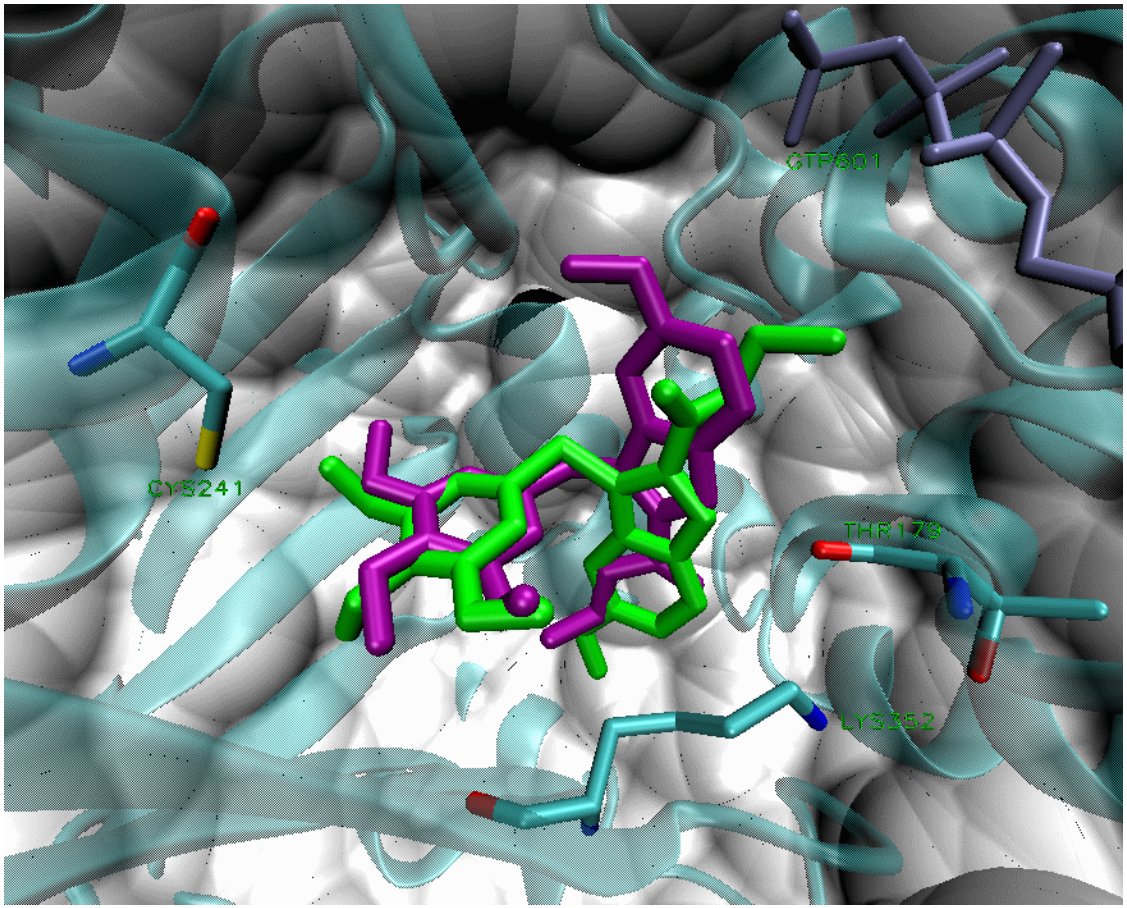
Arylthioindoles: 1st Series

Docking studies

- Semi-flexible docking studies (incremental construction algorithm) were performed using FlexX module in SYBYL 7.0 into the colchicine binding site of tubulin (PDB code: 1SA0).
- All the minimizations were performed with MOE until RMSD gradient of 0.05 kcal mol^{-1} $Å^{-1}$ was reached with the MMFF94x force field. The partial charges were automatically calculated.
- Best docked structures were selected when there was a good overlapping of the trimethoxyphenyl group of DAMA-colchicine with that of the docked compound.

Arylthioindoles: 1st Series

Docking studies



MOE and SYBYL binding mode

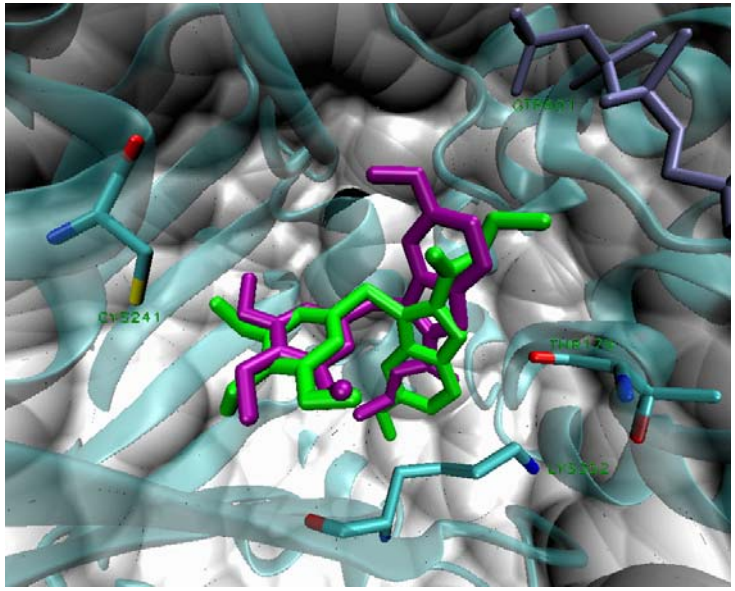
Arylthioindoles: 1st Series

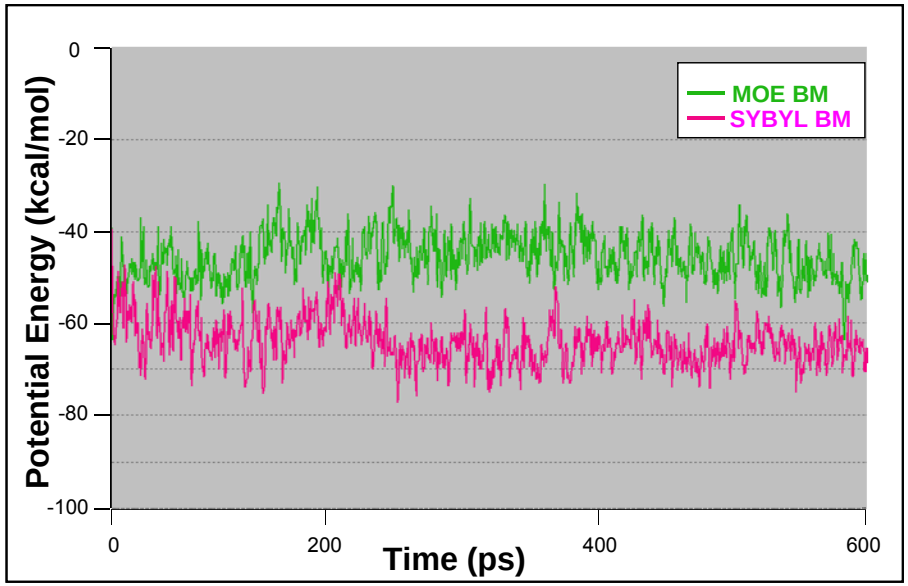
Molecular dynamics studies

- Molecular dynamics was performed with MOE 2004.03 using the NVT environment for 600 ps and constant temperature of 300 K using the MMFF94x force field with a time step of 2 fs.
- Residues within 15 Å of the ligand were allowed to move freely, keeping the rest of the protein fixed.
- The binding site was soaked in a water sphere of 25 Å radius from the sulfur atom of the ligand, and the total charge of the system included in the water droplet did not require any adjustment.

Arylthioindoles: 1st Series

Molecular dynamics studies

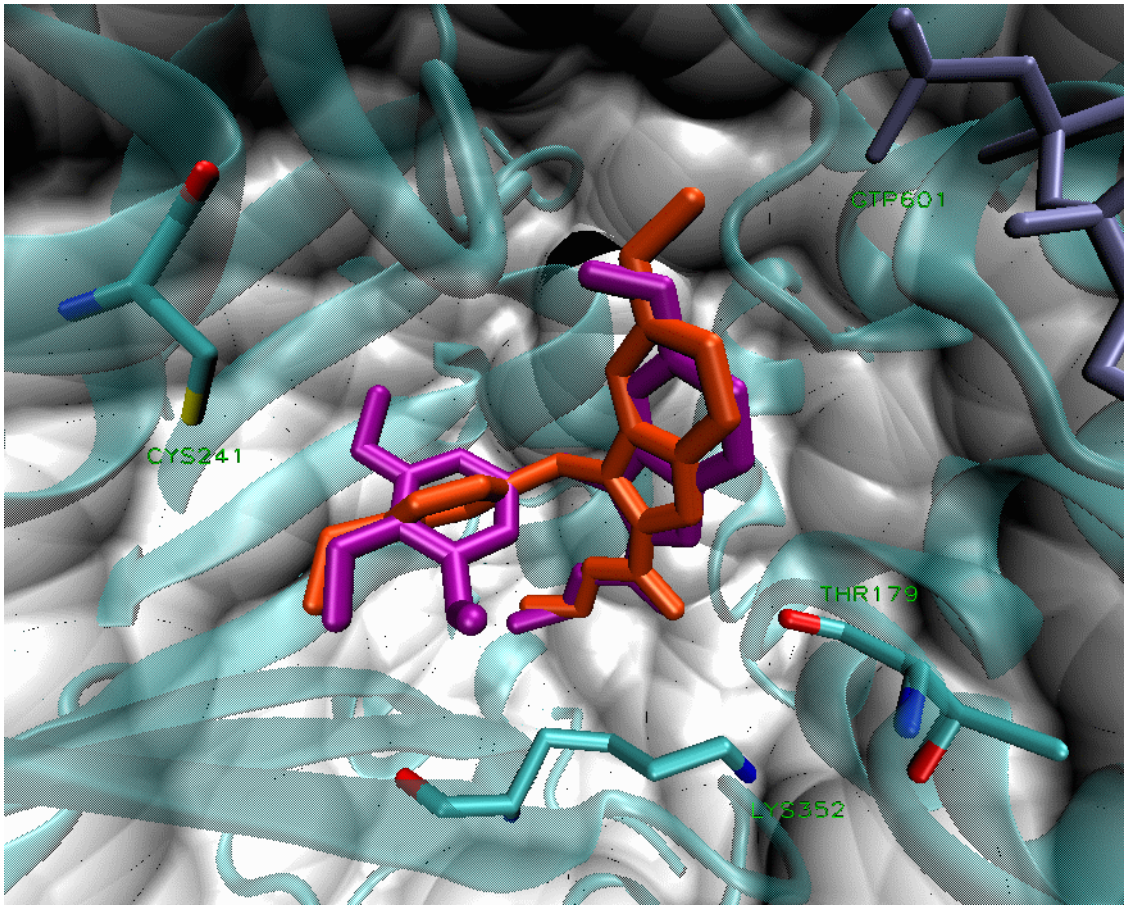




MD revealed a difference of ≈ 20 kcal/mol between the two conformations

Arylthioindoles: 1st Series

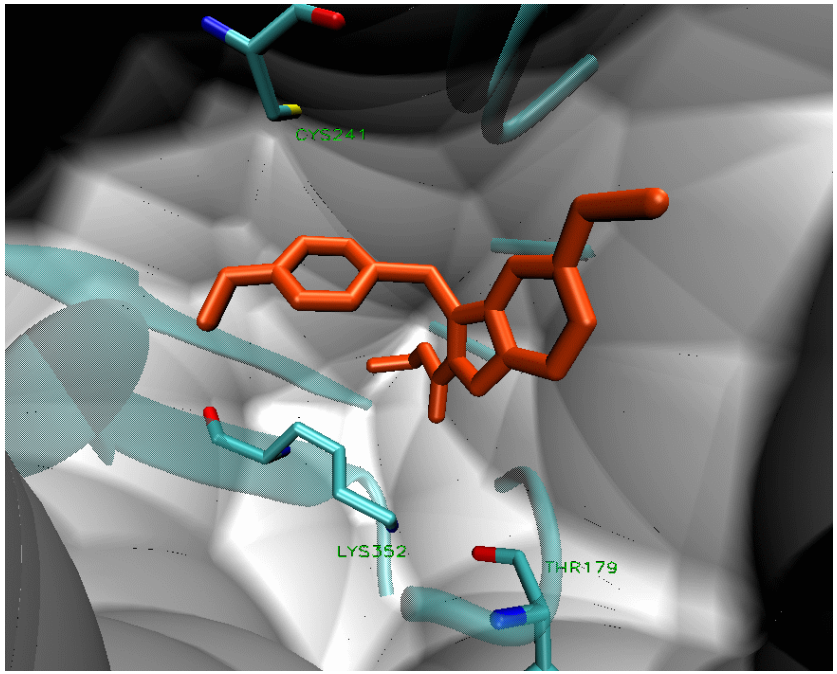
Molecular dynamics studies

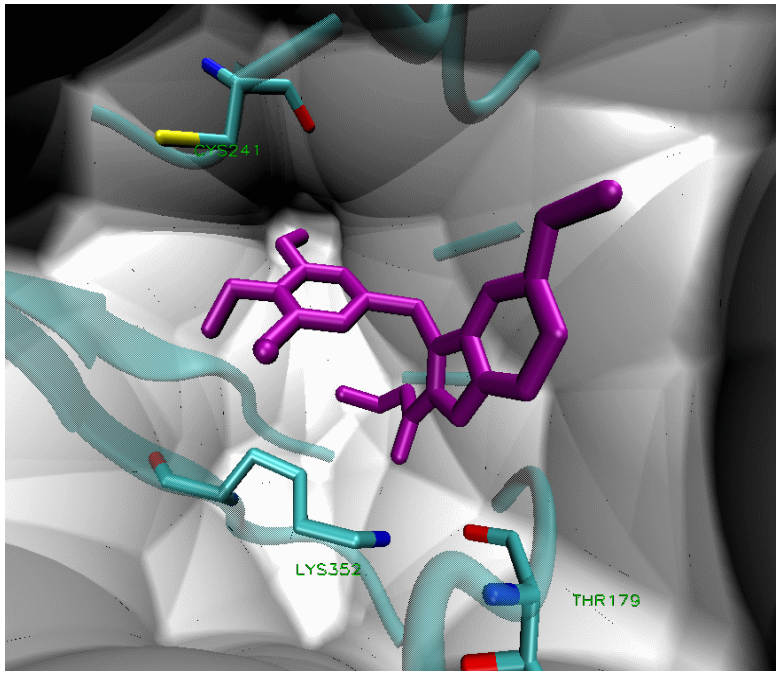


Cpd	IC_{50} ± SD (μM)	U_{ab} (kcal/mol)
4-OMe-ATI	>40	?
3,4,5-$(OMe)_3$-ATI	2.4 ± 0.2	?

Arylthioindoles: 1st Series

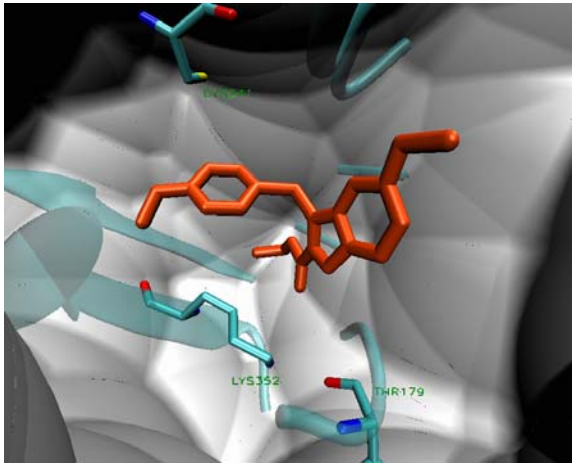
Molecular dynamics studies

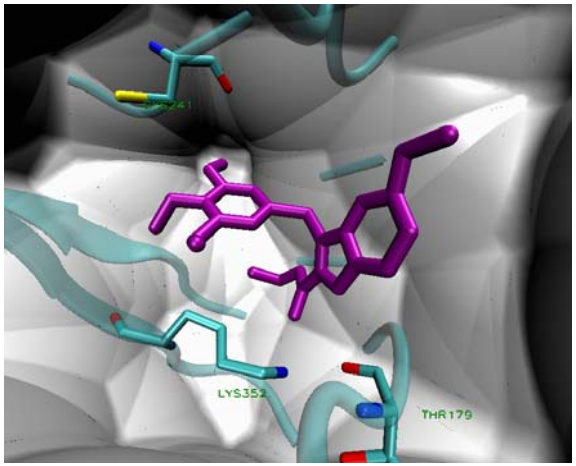


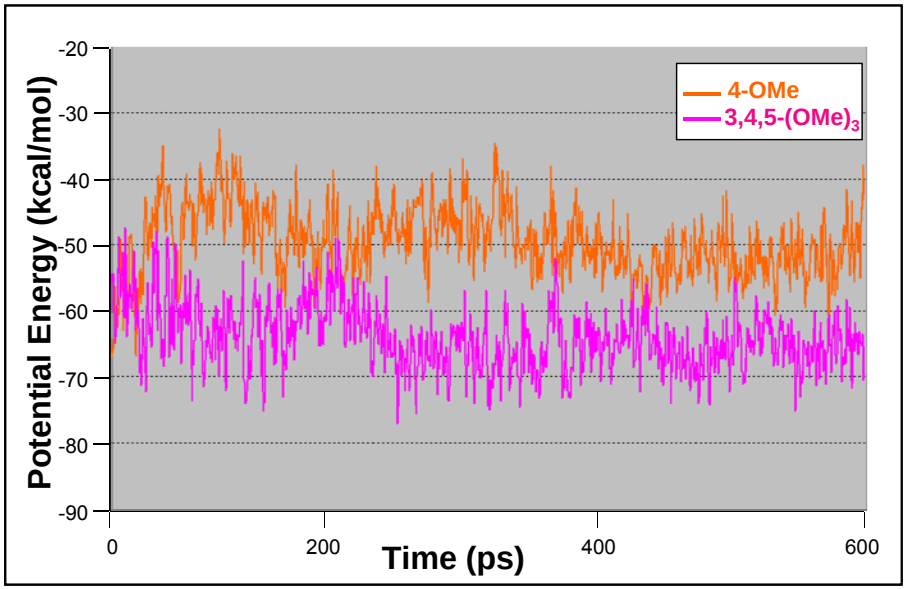


Arylthioindoles: 1st Series

Molecular dynamics studies



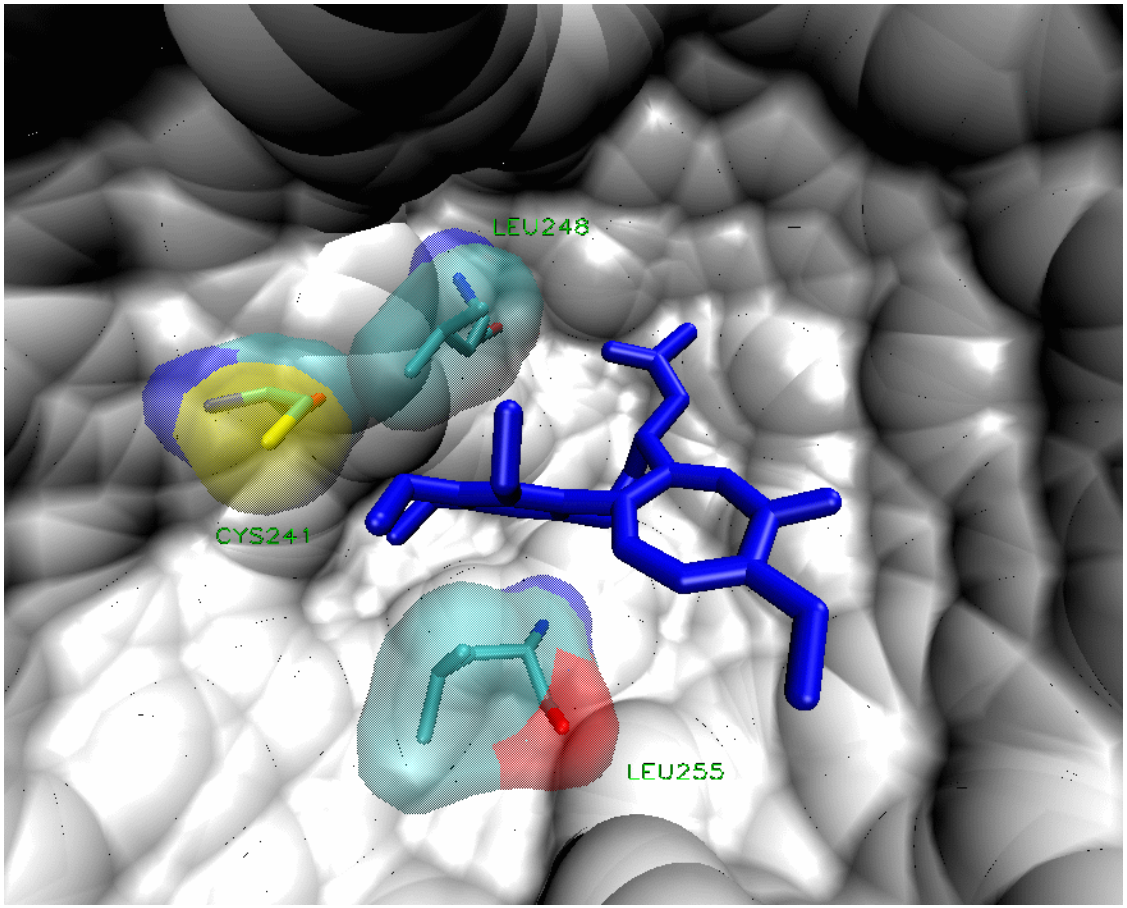




Cpd	IC_{50} ± SD (μM)	U_{ab} (kcal/mol)
4-OMe-ATI	>40	– 49.8
3,4,5-$(OMe)_3$-ATI	2.4 ± 0.2	– 64.7

Arylthioindoles: 1st Series

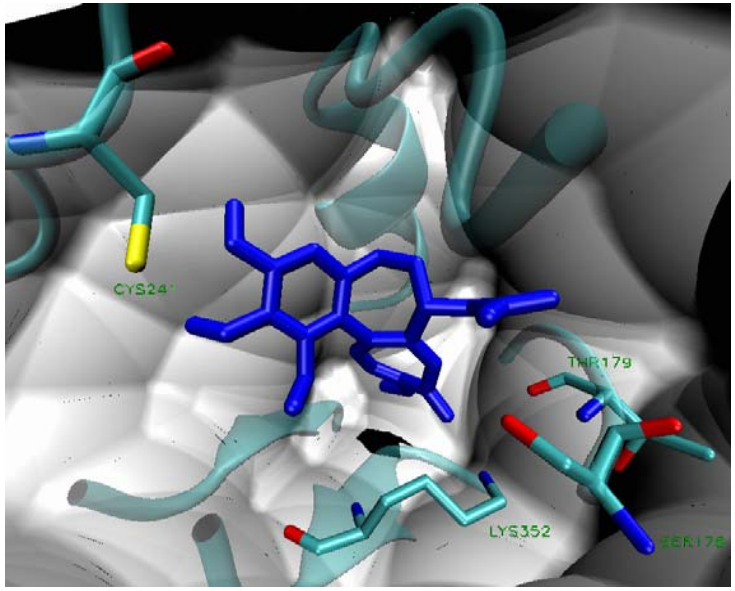
Molecular dynamics studies

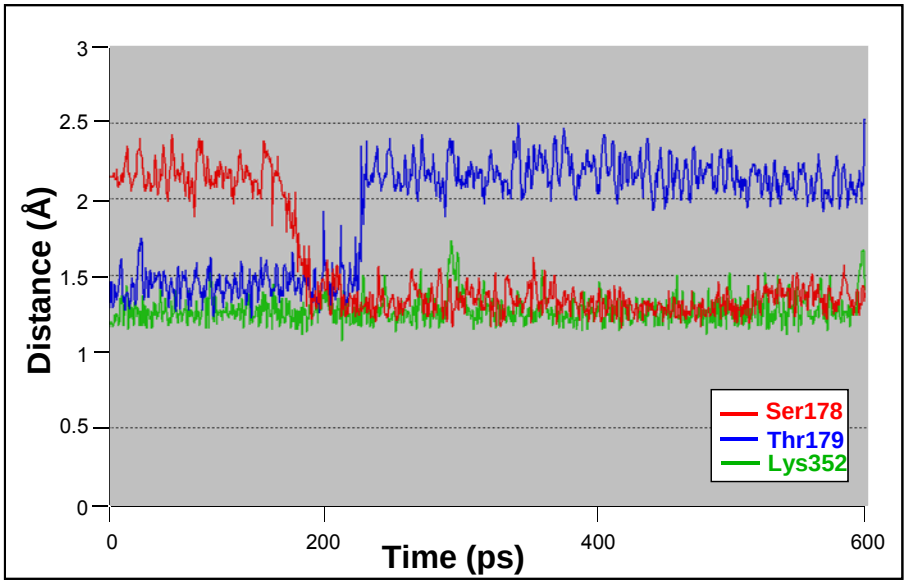


Colchicine binding mode

Arylthioindoles: 1st Series

Molecular dynamics studies

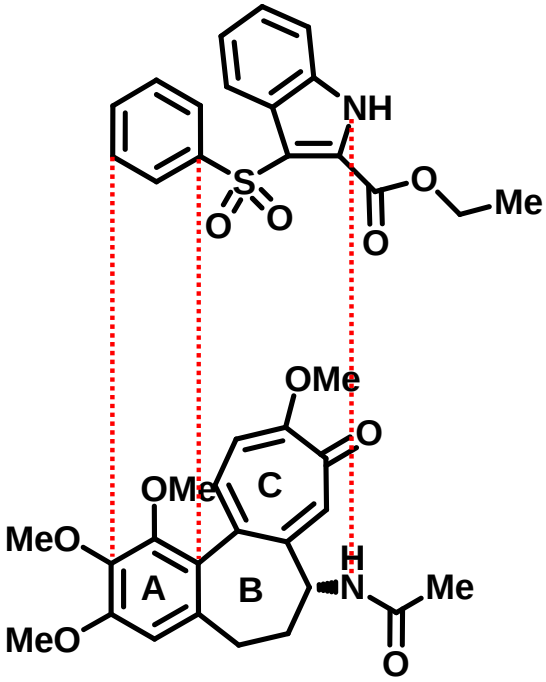




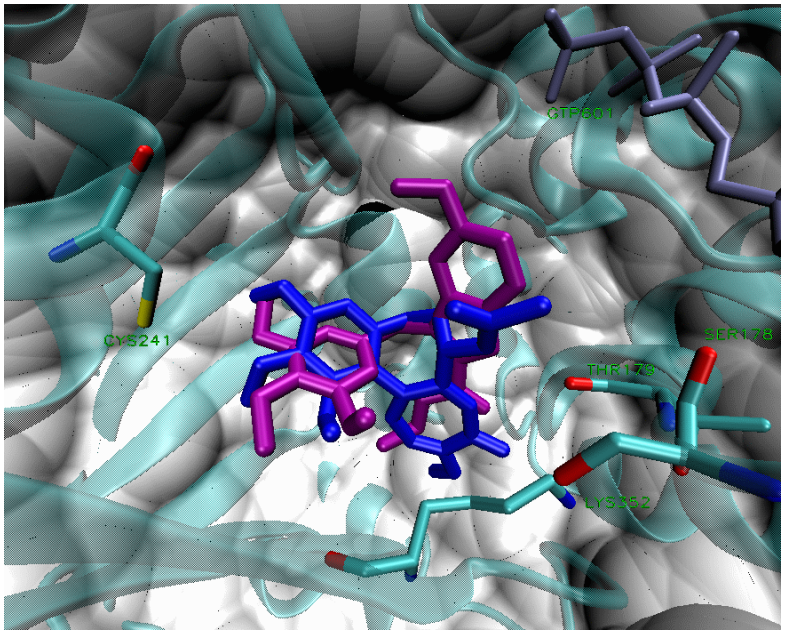
Arylthioindoles: 1st Series

Molecular dynamics studies

Hit compound

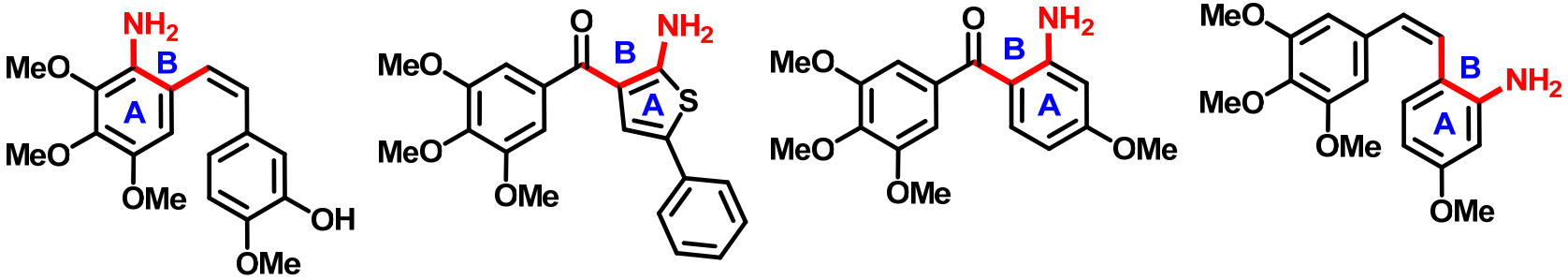


Colchicine

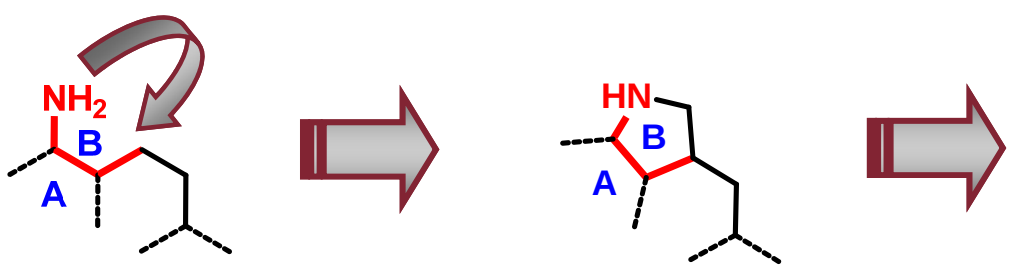


Arylthioindoles: 2nd Series

Rational



J. Med. Chem. **2002**, *45*, 2556-2562. *J. Med. Chem.* **2006**, *49*, 6412-6415. *J. Med. Chem.* **2006**, *49*, 3906-3915. *J. Med. Chem.* **2006**, *49*, 6425-6428.



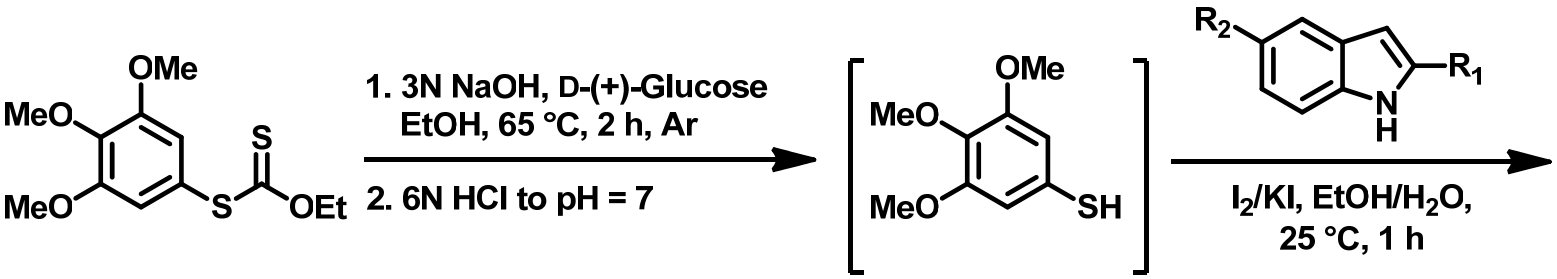
19 new ATIs

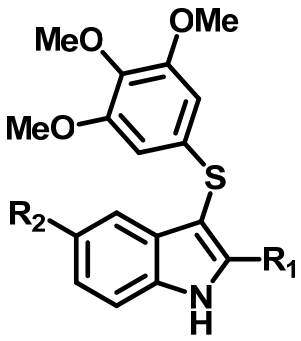
R_1 = H, Me; R_2 = H, F, Cl, Br, I, NO_2, NH_2, OH, OMe, OEt, O-*i*-Pr, $O(CH_2)_2OCH_2Ph$.

J. Med. Chem. **2007**, *50*, 2865-2874.

Arylthioindoles: 2nd Series

Synthesis

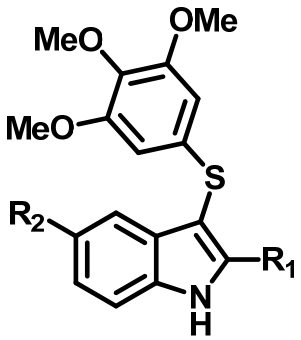




40-55%

Arylthioindoles: 2nd Series

Biological activity



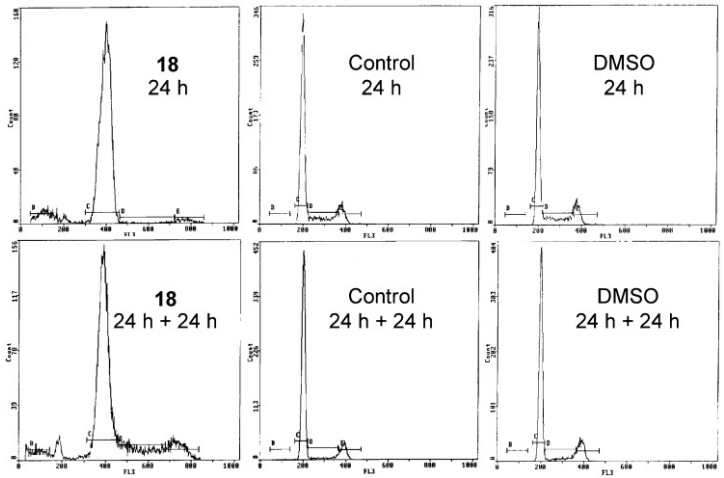
10, 14-23

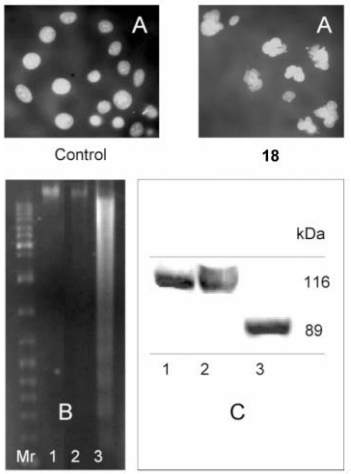
Cpd	R_1	R_2	Tubulin[a] IC_{50} ± SD (μM)	MCF-7[b] IC_{50} ± SD (nM)	Colchicine binding[c] (% ± SD)
14	H	H	2.6 ± 0.2	34 ± 9	68 ± 0.8
15	Me	H	6.8 ± 0.6	46 ± 4	61 ± 4
16	H	Cl	2.6 ± 0.2	77 ± 7	51 ± 4
17	Me	Cl	2.7 ± 0.5	82 ± 10	59 ± 5
18	H	Br	1.6 ± 0.3	43 ± 7	65 ± 3
19	H	NO_2	16 ± 0.4	560 ± 70	-
20	H	Me	2.7 ± 0.2	16 ± 6	56 ± 3
21	H	OMe	4.1 ± 0.6	22 ± 2	61 ± 4
22	Me	OMe	3.3 ± 0.2	18 ± 4	69 ± 0.2
23	H	OEt	2.1 ± 0.1	16 ± 5	76 ± 5
10	COOMe	OMe	2.0 ± 0.2	13 ± 3	90 ± 1
Colch[d]			3.2 ± 0.4	13 ± 3	-
CSA4[e]			2.2 ± 0.2	17 ± 10	97 ± 0.5

[a]Inhibition of tubulin polymerization. [b]Inhibition of growth of MCF-7 human breast carcinoma cells. [c]Inhibition of [^{3}H]colchicine binding. [d]Colchicine. [e]Combretastatin A4.

Arylthioindoles: 2nd Series

Biological activity



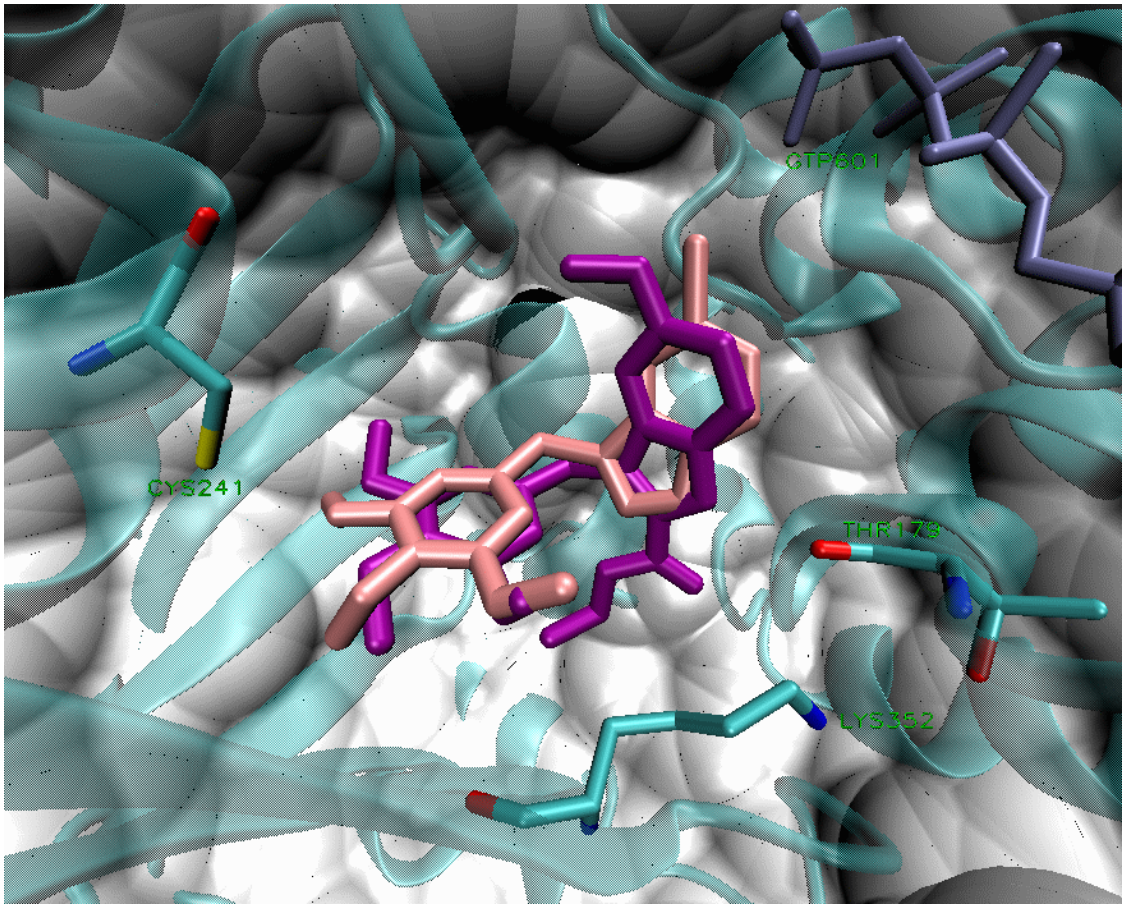


Cell cycle phase	18[a]	Control 24 h	DMSO[b]	18[c]	Control 24 h + 24h	DMSO
A0[d]	6.5	0.2	0.1	4.5	0.4	0.2
G1	1.8	69.6	73.9	3.5	81.4	76.7
S	8.9	17.4	15.2	10.5	6.3	8.1
G2/M	56.1	12.0	10.1	53.1	11.5	14.4
>4C	4.2	0	0	26.0	0	0

Data are expressed as % of cells in each cell cycle phase. A typical experiment is shown. [a]Cells were treated with **18** at 10 μM for 24 h. [b]Parallel samples incubated with 0.1% DMSO (the same final concentration used with **18** at 10 μM) did not significantly alter cell cycle distribution. [c]Cells were further incubated in drug-free medium for 24 h. [d]Indicates cells with a sub-G1 DNA content, probably representing a small population of apoptotic cells.

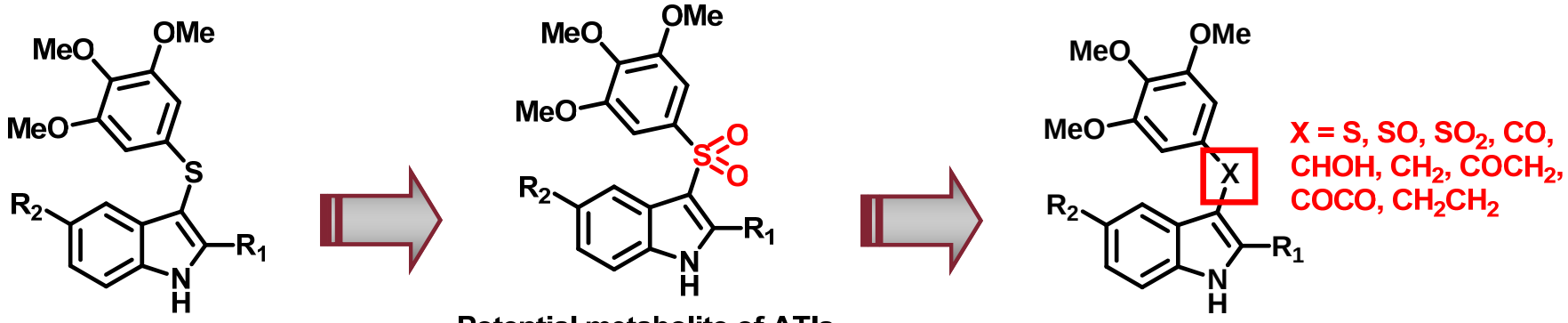
Arylthioindoles: 2nd Series

Molecular modelling studies



Arylthioindoles: 3rd Series

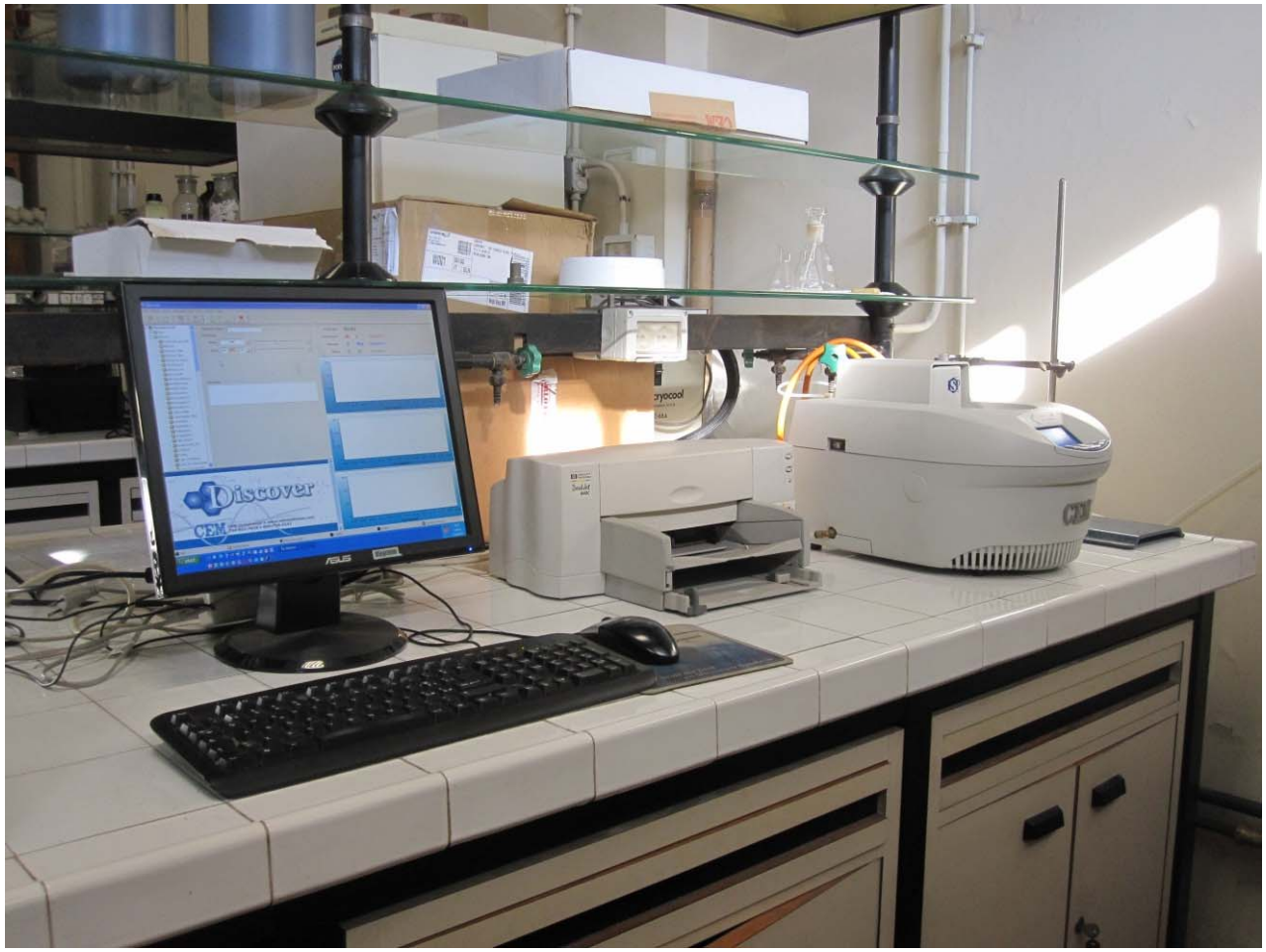
Rational



J. Med. Chem. **2009**, *52*, 7512-7527.

Arylthioindoles: 3rd Series

Discover SP focused microwave reactor @ DDSC



Arylthioindoles: 3rd Series

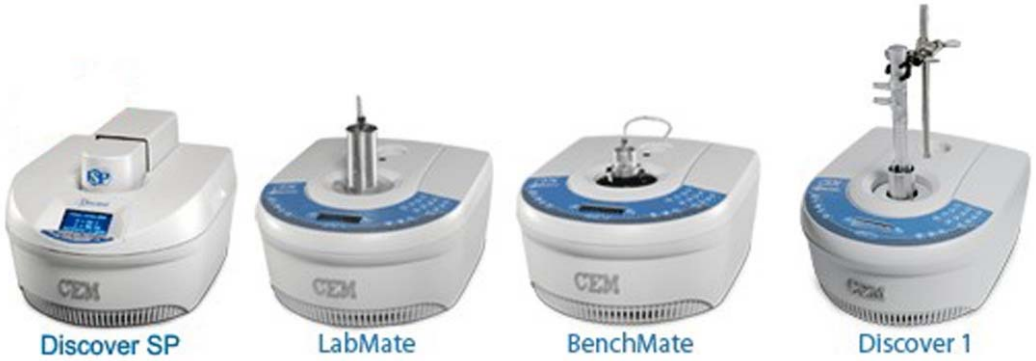
Discover SP focused microwave reactor @ DDSC

Closed vessel mode

Open vessel mode

Arylthioindoles: 3rd Series

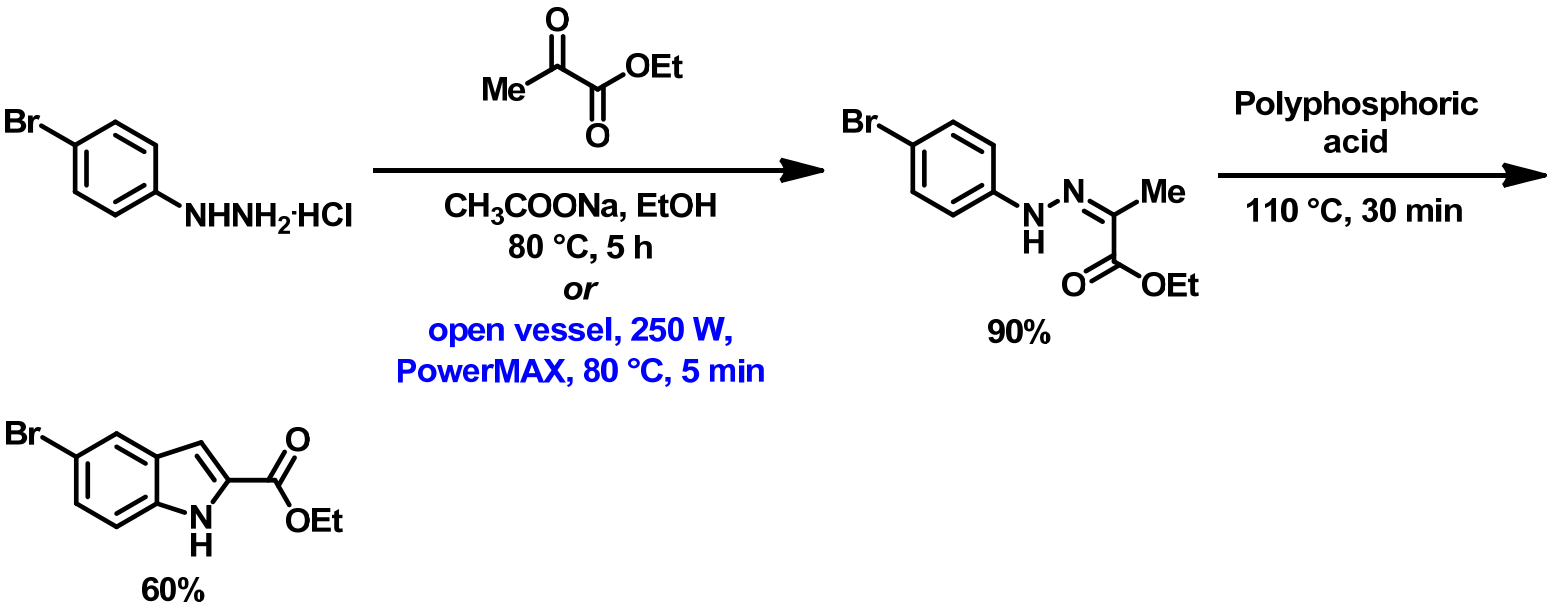
Discover SP focused microwave reactor @ DDSC



- Focused single-mode and self-tuning cavity
- 1-300 W power supply
- Vertically-focused IR temperature sensor
- Open (≤100 mL) and closed (10 and 35 mL) vessel reactions
- Simultaneous cooling while heating (PowerMax technology)
- Simultaneous venting while heating (Activent technology)

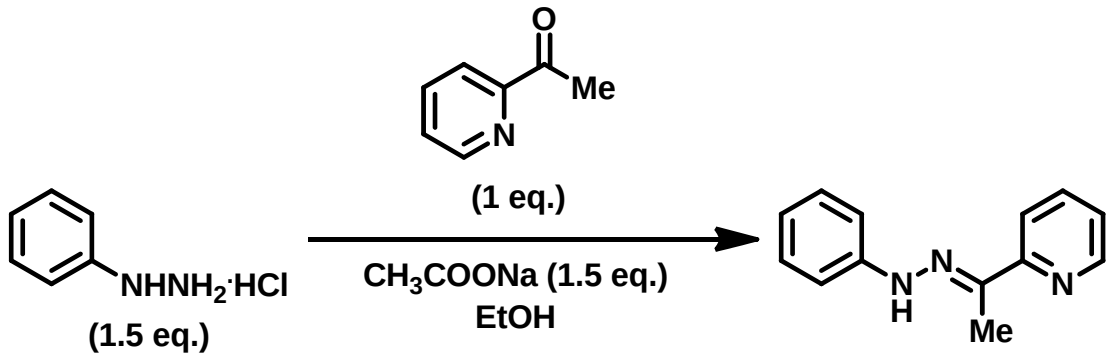
Arylthioindoles: 3rd Series

Synthesis



Arylthioindoles: 3rd Series

Synthesis



Heating	Mode	Temp (°C)	Ramp Time	Hold Time	Power (W)	MaxPress[a] (PSI)	PowerMax[b]	Yield[c] (%)
Oil bath		80		5 h				90
MW	Closed vessel	80	1 min	2 min	50	250	Off	15
MW	Closed vessel	80	1 min	2 min	100	250	On	23
MW	Closed vessel	110	1 min	2 min	125	250	Off	10
MW	Closed vessel	130	1 min	4 min	150	250	Off	3
MW	Open vessel	80	1 min	5 min	250		Off	50
MW	Open vessel	80	1 min	5 min	250		On	98

[a]Maximum pressure. [b]Simultaneous cooling while heating. [c]Isolated yield.

Arylthioindoles: 3rd Series

Synthesis

Reaction

221 Idrazone Ope User: MicrowaveLab5 Open Vessel Snap Cap

Method Run

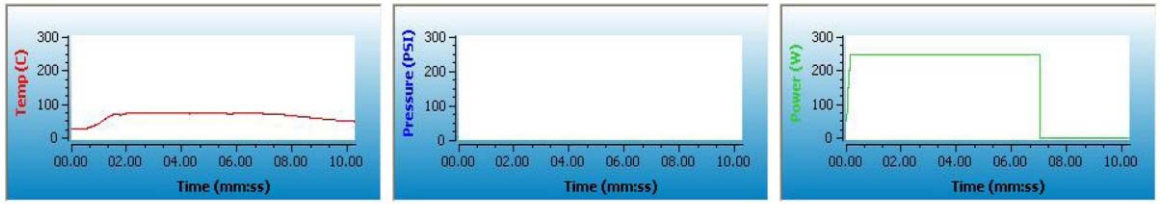
Name: Idrazone Ope

Type: Dynamic

Prestirring(mm:ss):	00:15

Stage	Temp(C)	Time(mm:ss)	Pressure(PSI)	Power(W)	PowerMAX	Stirring
1	80	05:00	250	250	Yes	High

Graphs



Reaction Notes

Reaction started:	15/01/2010 11.03.13
Reaction cooling started:	15/01/2010 11.10.14

Reaction Completed Successfully!

Maximum temperature:	76 C
Maximum pressure:	0 PSI
Time to try to obtain setpoint:	07:01 mm:ss

Arylthioindoles: 3rd Series

Synthesis

ACS Combinatorial Science

LETTER

pubs.acs.org/acscombsci

Open Vessel and Cooling while Heating Microwave-Assisted Synthesis of Pyridinyl *N*-Aryl Hydrazones

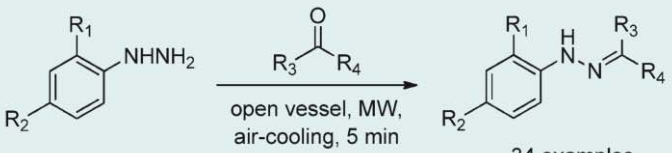
Giuseppe La Regina,* Valerio Gatti, Francesco Piscitelli, and Romano Silvestri

Istituto Pasteur, Fondazione Cenci Bolognetti, Dipartimento di Chimica e Tecnologie del Farmaco, Sapienza Università di Roma, Piazzale Aldo Moro 5, I-00185, Rome, Italy

Supporting Information

ABSTRACT: We reported the first example of open vessel and cooling while heating microwave-assisted synthesis of pyridinyl *N*-aryl hydrazones. Compounds were prepared in excellent isolated yields (88−98%) in only 5 min, by reacting 4- and 2,4-(di)substituted phenylhydrazines, bearing both electron-donating (4-CH_3, 4-OCH_3) and -withdrawing (4-Cl, 4-Br, 4-CF_3, 4-NO_2, 2,4-Cl_2) groups with 2-, 3-, and 4-acetylpyridine. The method was successfully extended to other carbonyl compounds.

KEYWORDS: hydrazones, microwave synthesis, cooling while heating, open vessel

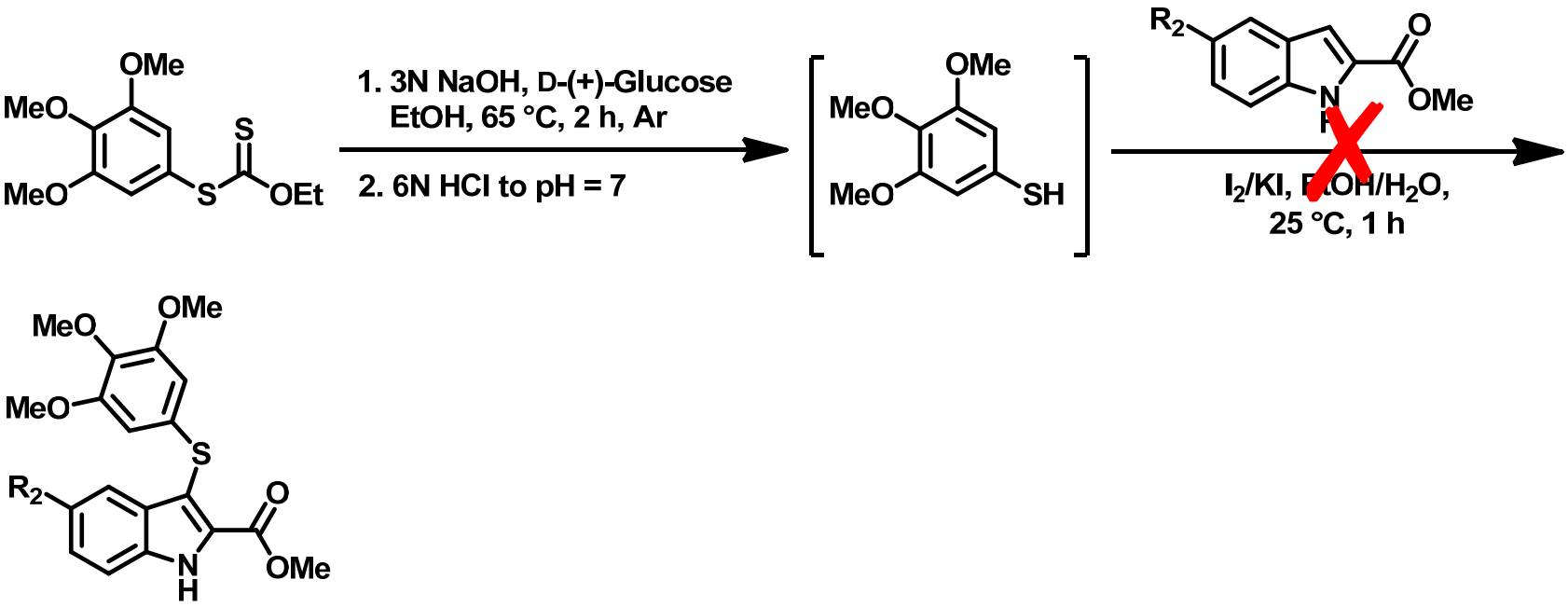


34 examples
88-98%

R_1 = H, Cl; R_2 = H, CH_3, OCH_3, Cl, Br, CF_3, NO_2, R_3 = H, CH_3; R_4 = phenyl, naphthalen-2-yl, pyrrol-2-yl, furan-2-yl, thiophen-2-yl, pyridin-2-yl, pyridin-3-yl, pyridin-4-yl

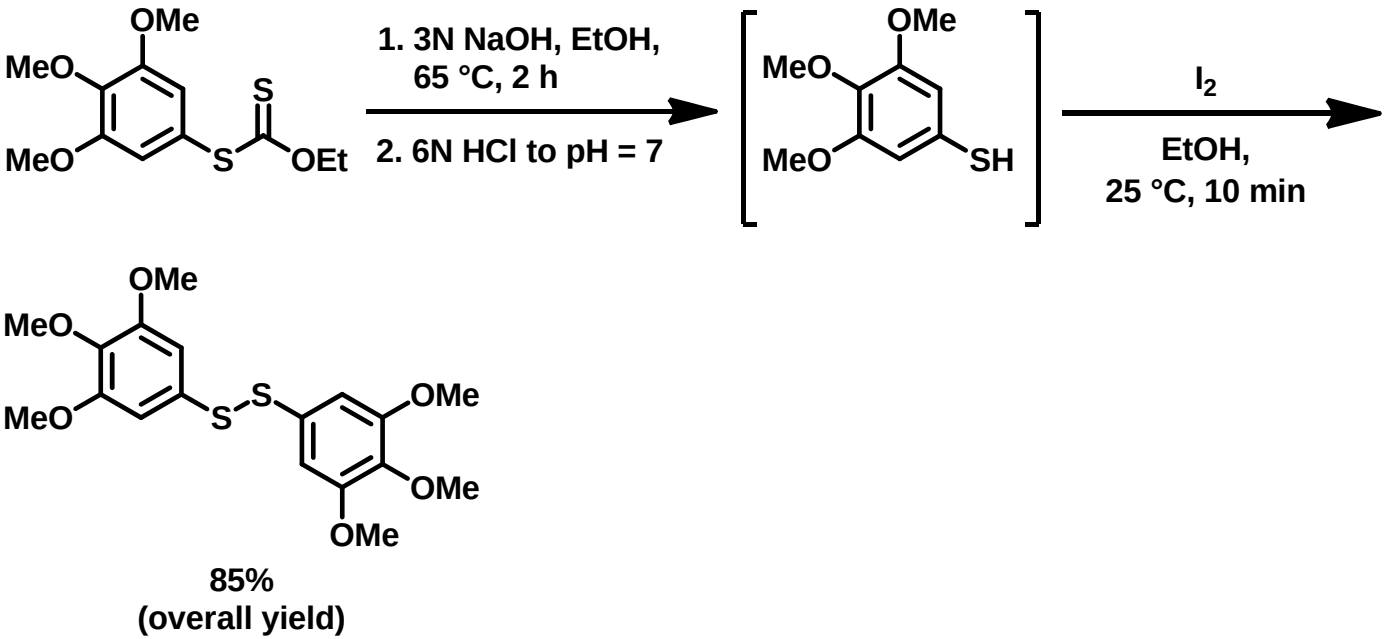
Arylthioindoles: 3rd Series

Synthesis



Arylthioindoles: 3rd Series

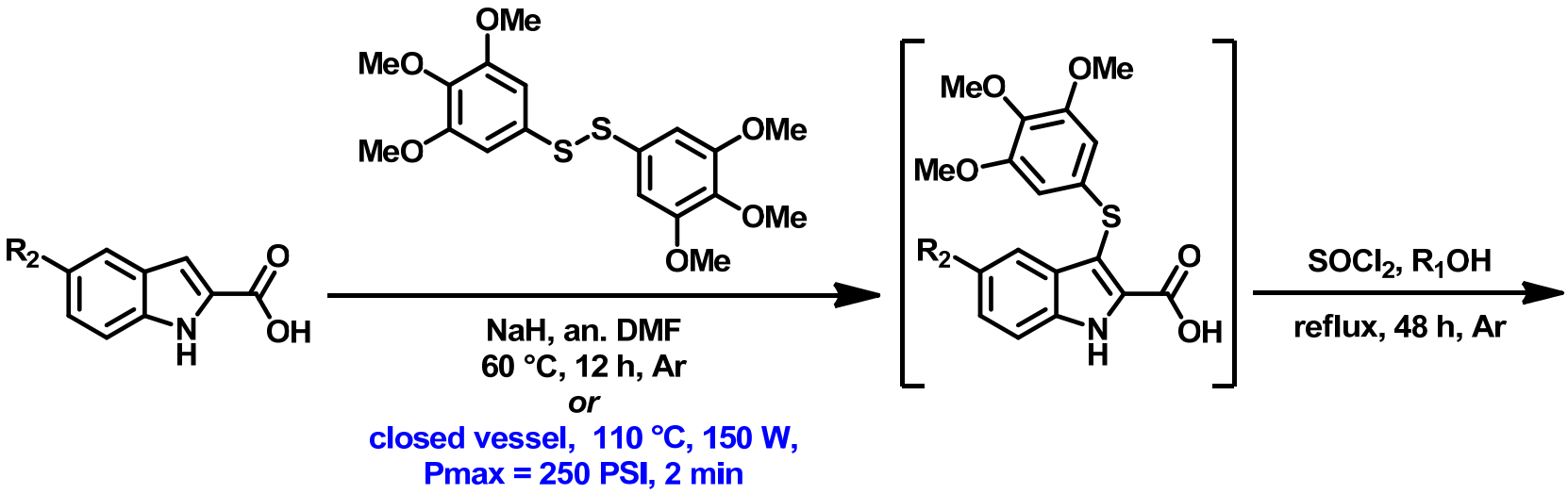
Synthesis

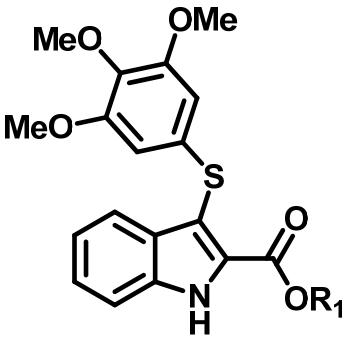


85%
(overall yield)

Arylthioindoles: 3rd Series

Synthesis

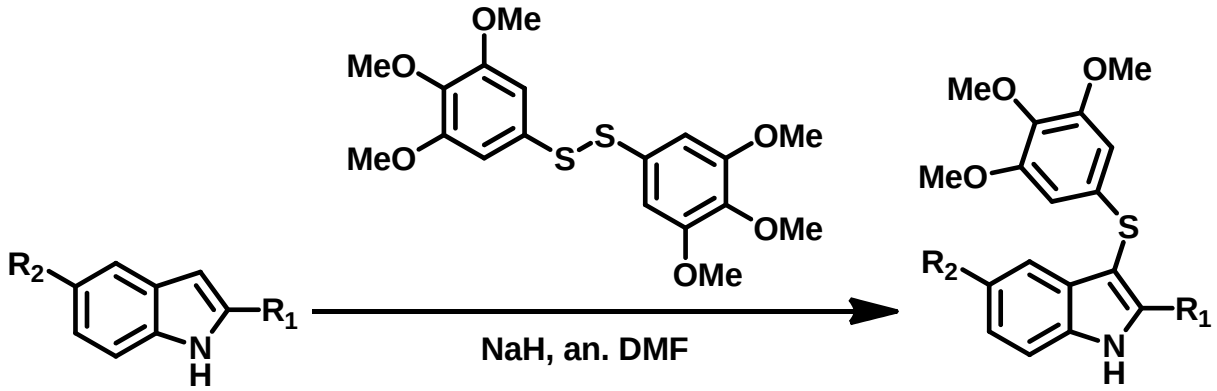




20-30% *or* 25-35%

Arylthioindoles: 3rd Series

Synthesis

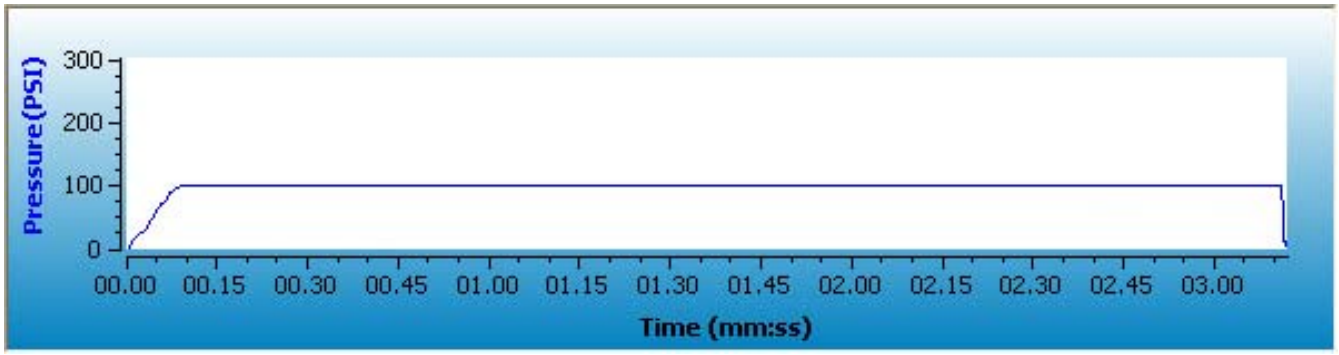


Heating	Temp (°C)	Ramp Time	Hold Time	Power (W)	Activent[a]	Yield[b] (%)
Oil bath	60	-	12 h	-	-	90
MW	100	1 min	2 min	70	Off	35
MW	130	1 min	2 min	100	Off	45
MW	160	1 min	2 min	130	Off	40
MW	130	1 min	4 min	100	Off	25
MW	130	1 min	8 min	100	Off	15
MW	100	1 min	2 min	90	On	65
MW	130	1 min	2 min	120	On	98

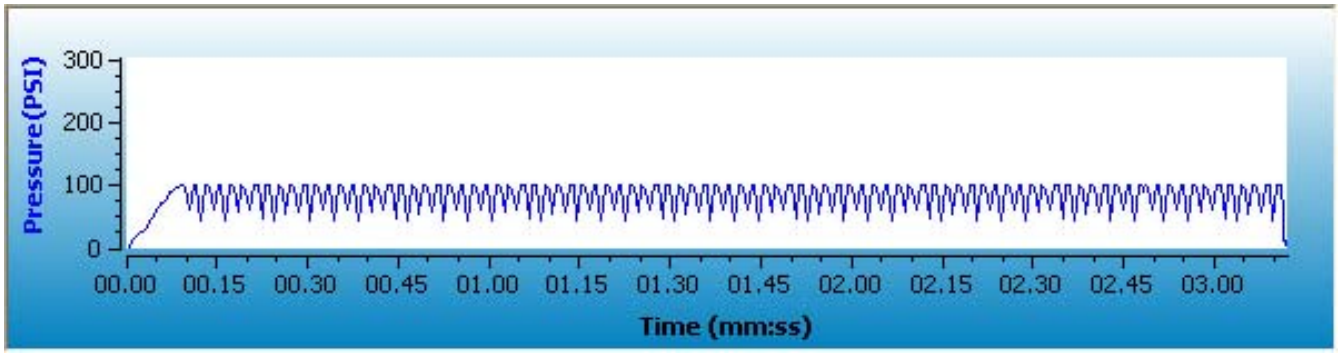
[a]Venting while heating. [b]Isolated yield.

Arylthioindoles: 3rd Series

Synthesis



Closed vessel, 130 ºC, 120 W, 2 min, 45%



Closed vessel, 130 ºC, 120 W, 2 min, Activent, 98%

Arylthioindoles: 3rd Series

Synthesis

ACS Combinatorial Science

Letter

pubs.acs.org/acscombsci

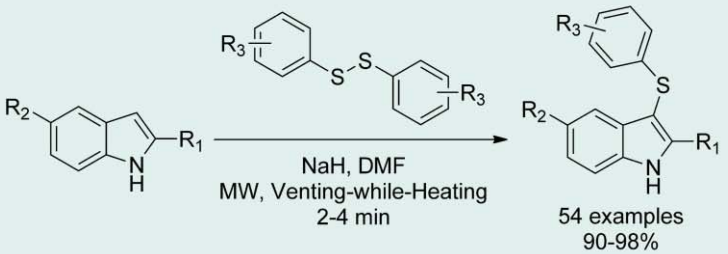
Venting-while-Heating Microwave-Assisted Synthesis of 3-Arylthioindoles

Giuseppe La Regina,* Valerio Gatti, Valeria Famiglini, Francesco Piscitelli, and Romano Silvestri

Istituto Pasteur-Fondazione Cenci Bolognetti, Dipartimento di Chimica e Tecnologie del Farmaco, Sapienza Università di Roma, Piazzale Aldo Moro 5, I-00185, Rome, Italy

Supporting Information

ABSTRACT: We report the first example of venting-while-heating microwave-assisted synthesis of a small library of 3-arylthioindoles. Compounds were prepared in excellent isolated yields (90−98%) within 4 min in a closed vessel by treating indoles with disulfides in the presence of sodium hydride in anhydrous *N*,*N*-dimethylformamide. The method was not affected by electron-donating and -withdrawing substituents both on 3-arylthio moiety and at 2- and 5-positions of the indole nucleus.

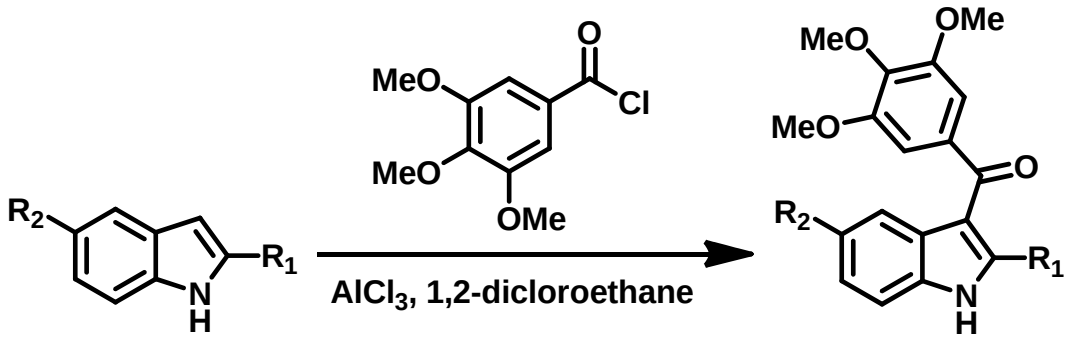


R_1 = H, Me, Ph, COOH, COOEt; R_2 = H, Cl, OMe; R_3 = H, Cl, OMe, 2,4-Cl_2, 2,5-OMe_2, 3,4,5-OMe_3

KEYWORDS: *microwave-assisted organic synthesis, dielectric heating, venting while heating, sulfenylation, 3-arylthioindoles, indole*

Arylthioindoles: 3rd Series

Synthesis



Heating	Mode	Temp (°C)	Ramp Time	Hold Time	Power (W)	Press Max[a] (PSI)	PowerMax[b]	Yield[c] (%)
Oil bath	-	Reflux	-	2h	-	-	-	20
Oil bath	-	Reflux	-	12 h	-	-	-	18
Oil bath	-	Reflux	-	24 h	-	-	-	26
MW	Closed vessel	80	1 min	2 min	50	250	Off	20
MW	Closed vessel	80	1 min	2 min	100	250	On	19
MW	Closed vessel	110	1 min	2 min	150	250	Off	68
MW	Closed vessel	110	1 min	4 min	150	250	Off	50
MW	Closed vessel	110	1 min	8 min	150	250	Off	10
MW	Closed vessel	150	1 min	10 min	150	250	Off	2

[a]Maximum pressure. [b]Simultaneous cooling while heating. [c]Isolated yield.

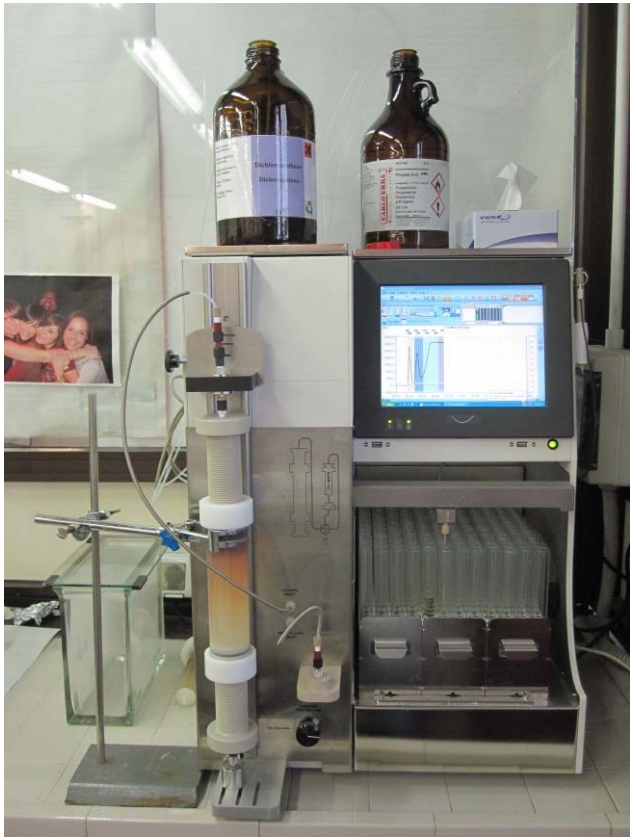
Arylthioindoles: 3rd Series

Spot II Flash flash chromatography system @ DDSC

Arylthioindoles: 3rd Series

Spot II Flash flash chromatography system @ DDSC

25 g silica gel column



80 g silica gel column

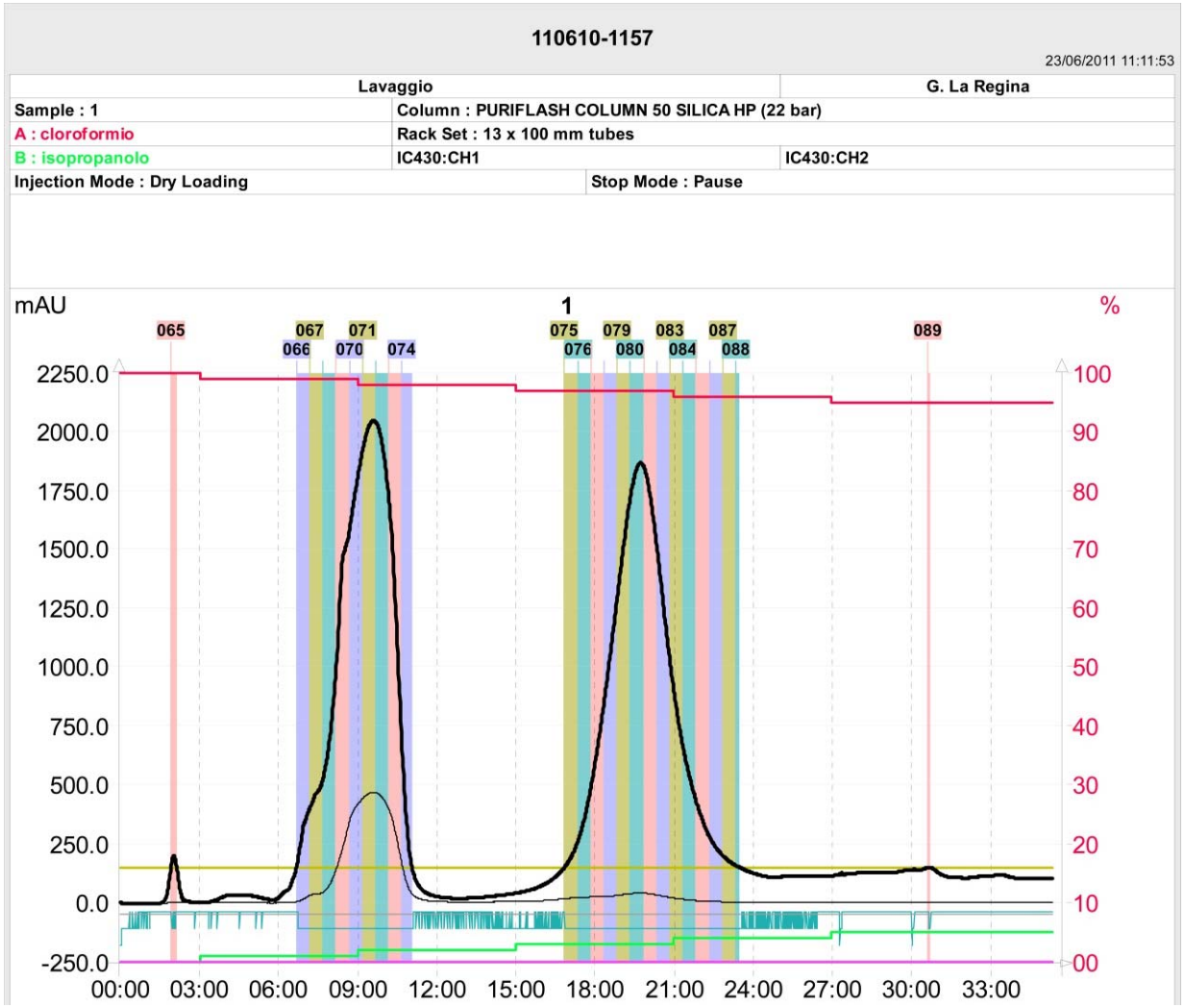
Arylthioindoles: 3rd Series

Spot II Flash flash chromatography system @ DDSC

- Panel PC with digital 10,4” touch screen
- Dual piston pump quaternary gradient + 1 inlet for air purge (35 bar maximum pressure, 250 mL/min)
- Isocratic, linear, auto-step, predefined, four solvents quaternary gradients
- Liquid or solid sample injections
- UV-Vis dual WL spectrophotometer (200-600 nm) detection
- 2.5 g - 1500 g usable cartridges size
- 3 racks 18 mm tubes (192 tubes) FC trays

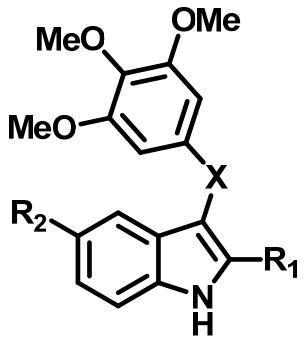
Arylthioindoles: 3rd Series

Spot II Flash flash chromatography system @ DDSC



Arylthioindoles: 3rd Series

Biological activity



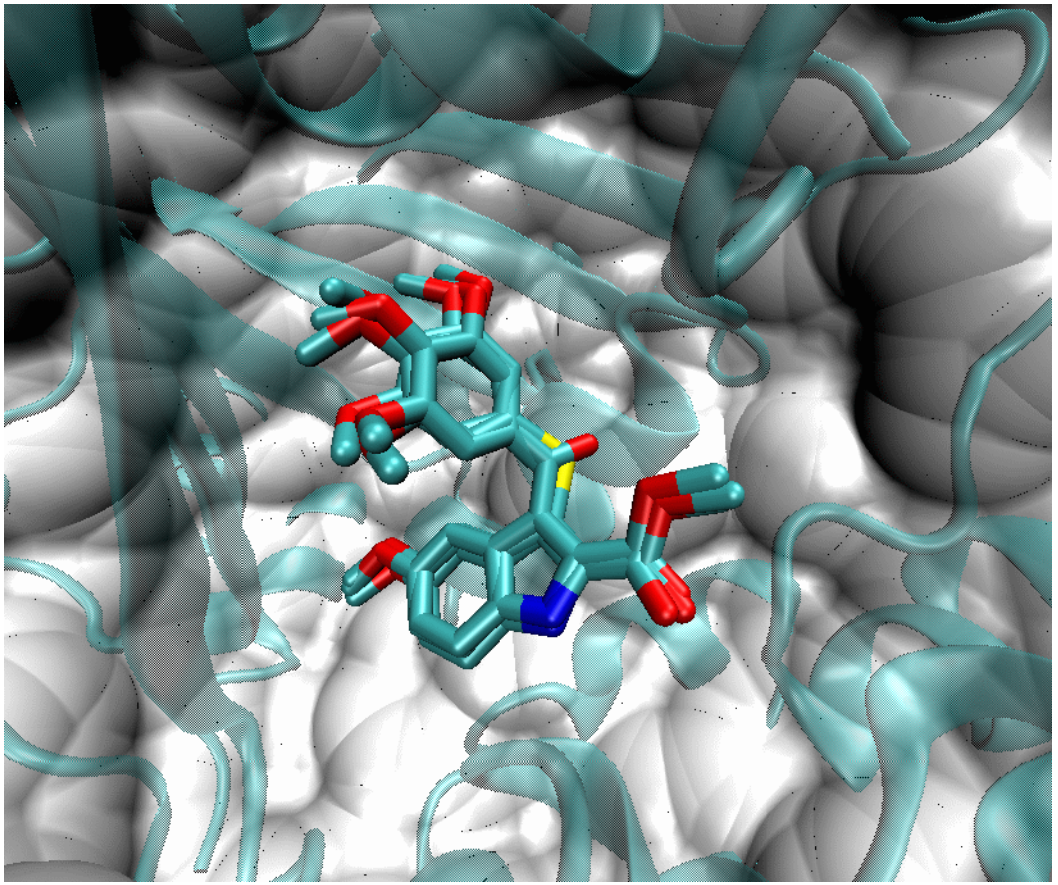
10, 24-37

Cpd	R_1	R_2	X	Tubulin[a] IC_{50} ± SD (μM)	MCF-7[b] IC_{50} ± SD (nM)	Colchicine binding[c] (% ± SD)
24	COOEt	Cl	$COCH_2$	>40	-	-
25	COOEt	Cl	COCO	>40	-	-
26	COOEt	Cl	CH_2CH_2	>40	-	-
27	H	Br	S	1.6 ± 0.3	43 ± 7	65 ± 3
28	H	Br	CO	1.9 ± 0.3	60 ± 0	45 ± 5
29	H	Br	CH_2	13 ± 0.8	-	-
30	COOMe	Br	S	0.99 ± 0.1	33 ± 10	75 ± 3
31	COOMe	Br	CO	1.3 ± 0.08	18 ± 4	67 ± 4
32	COOMe	Br	CH_2	1.3 ± 0.08	30 ± 9	59 ± 7
33	COOEt	Br	S	1.6 ± 0.2	83 ± 20	62 ± 7
34	COOEt	Br	CO	1.6 ± 0.05	67 ± 10	58 ± 2
35	COOEt	Br	CH_2	1.7 ± 0.2	100 ± 0	53 ± 4
36	COOMe	OMe	SO	>40	-	-
37	COOMe	OMe	SO_2	>40	-	-
10	COOMe	OMe	S	2.0 ± 0.2	13 ± 3	90 ± 1
Colch[d]				3.2 ± 0.4	13 ± 3	-
CSA4[e]				2.2 ± 0.2	17 ± 10	97 ± 0.5

[a]Inhibition of tubulin polymerization. [b]Inhibition of growth of MCF-7 human breast carcinoma cells. [c]Inhibition of [^{3}H]colchicine binding. [d]Colchicine. [e]Combretastatin A4.

Arylthioindoles: 3rd Series

Molecular modelling studies



Arylthioindoles: 4st Series

The present and the future

- Design of new potential inhibitors of tubulin polymerization with improved pharmacodynamic and pharmacokinetic properties.
- *In silico* screening of new compounds by docking studies into the colchicine binding site.
- Dynamics simulations of the best docked compound/tubulin complexes.
- Microwave-assisted of the best scored derivatives by open and closed vessel modes.
- Fully automated flash chromatography of the reaction mixtures.

Conclusions

Acknowledgments

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Conclusions

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“Mi sembra d’essere stato come un fanciullo sulla sponda del mare, che si diverte a raccogliere qualche sassolino levigato, una leggiadra conchiglia, mentre il grande oceano della verità mi si parava dinanzi tutto ancora inesplorato.”

I. Newton