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2-Hydroxy-4-methoxyacetophenone substituted chalcones: Synthesis and biological evaluation as potential anticancer agents

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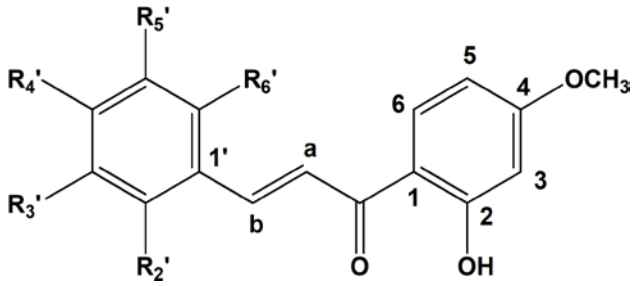
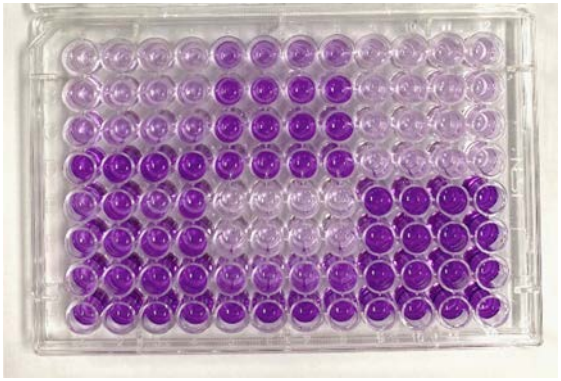


Synthesis of 2-hydroxy-4-methoxyacetophenone substituted chalcones

Cell Viability Assay



LY-2, LY-8 and LY-10 showed IC_{50} range of 4.6 to 9 µM on MCF-7, HT29 and A549 cancer cell lines

Abstract

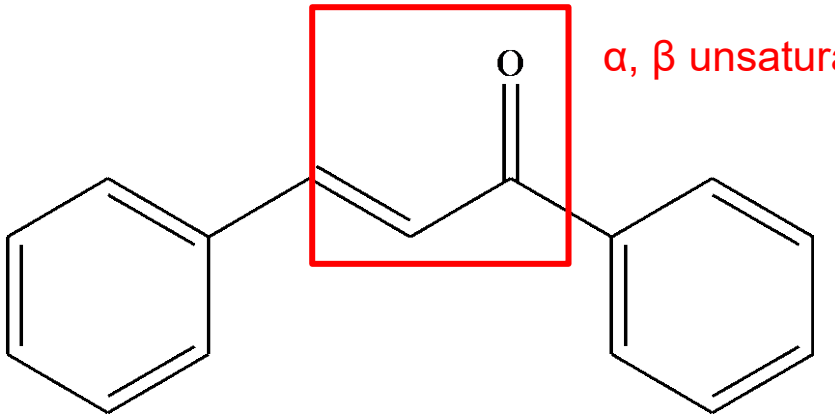
Cancer remains as the leading cause of death and targeted therapies are found unlikely to cure the disease, urging the development of new anticancer agents using “dirty” drugs. In recent years, chalcone derivatives have gained significant attention for their diverse biological activities, serving as good “dirty” drug candidates for the discovery of new leads. Motivated by studies that reported the promising anticancer activity of 2-hydroxy-4-methoxyacetophenone (HMA), we synthesised ten HMA substituted chalcones (LY-1 to LY-10) by reacting HMA with different substituted benzaldehydes using Claisen-Schmidt condensation. The synthesised compounds were purified using column chromatography and characterized by UV, IR, NMR and mass spectrometry. Cell viability assay was employed to evaluate the *in-vitro* anticancer activity of synthesised compounds against four human cancer cell lines, MCF-7 and MDA-MB-231 (human breast cancer), HT29 (human colorectal cancer) and A549 (human lung cancer) in comparison with doxorubicin as positive control. Three compounds (LY-2, LY-8 and LY-10) exhibited potent inhibitory activity against MCF-7, HT29 and A549 cancer cell lines with IC_{50} ranged from 4.61 to 9 μM. In addition, these compounds demonstrated minimal inhibition effect on non-cancerous human dermal fibroblast cells with IC_{50} more than 20 μM. These results call for further studies on active compounds to establish their possible development as promising anticancer drugs.

Keywords: Synthesis; Claisen-Schmidt condensation; Chalcones; Anticancer activity

Introduction

Chalcone

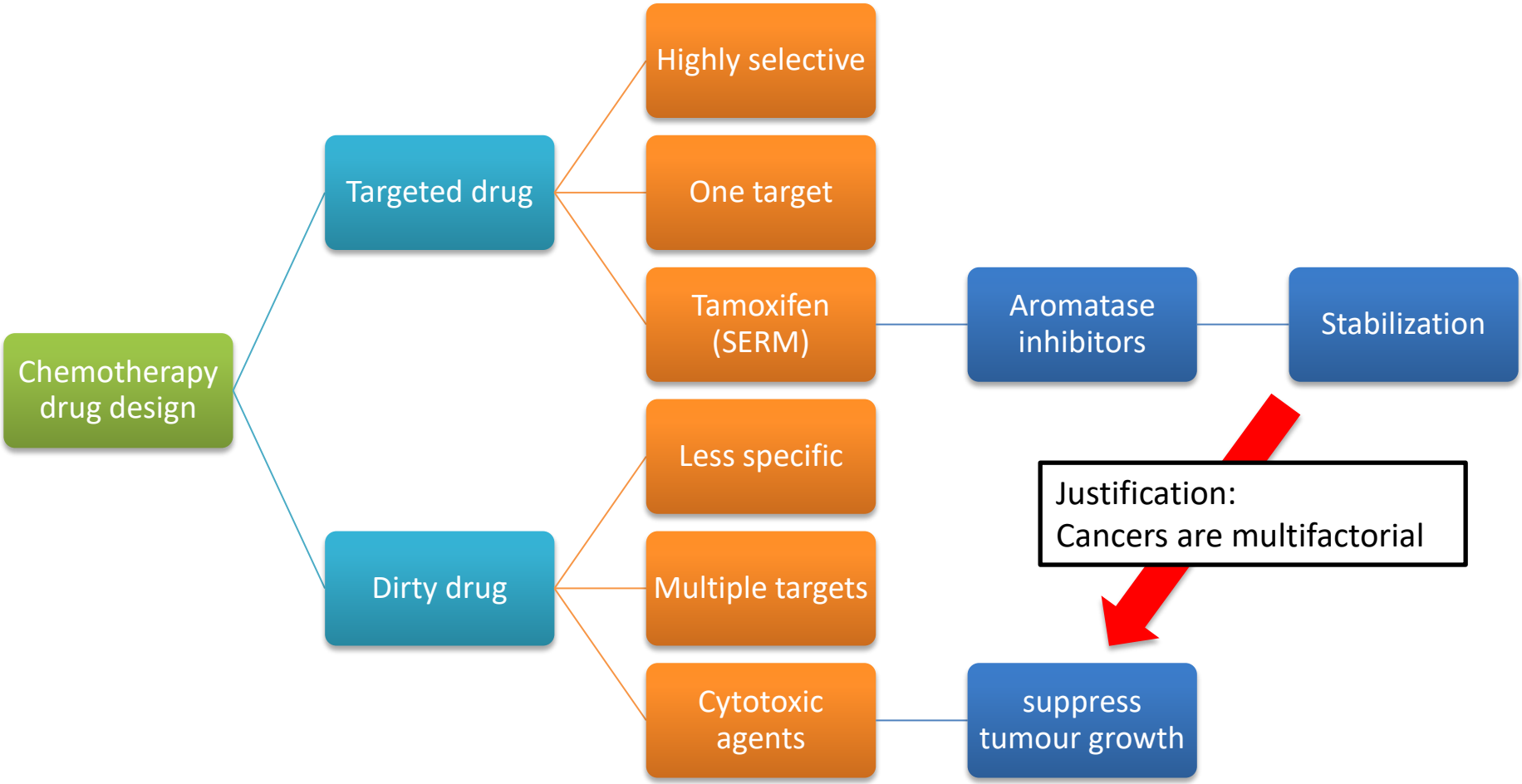
- Attractive drug design template
- IUPAC Name: 1, 3-diphenyl-2-propene-1-one
- Two aromatic rings linked by α, β unsaturated carbonyl bridge
- Wide spectrum biological activities: cytotoxicity, antioxidant, antibacterial, antifungal, anti-inflammatory etc



α, β unsaturated carbonyl bridge

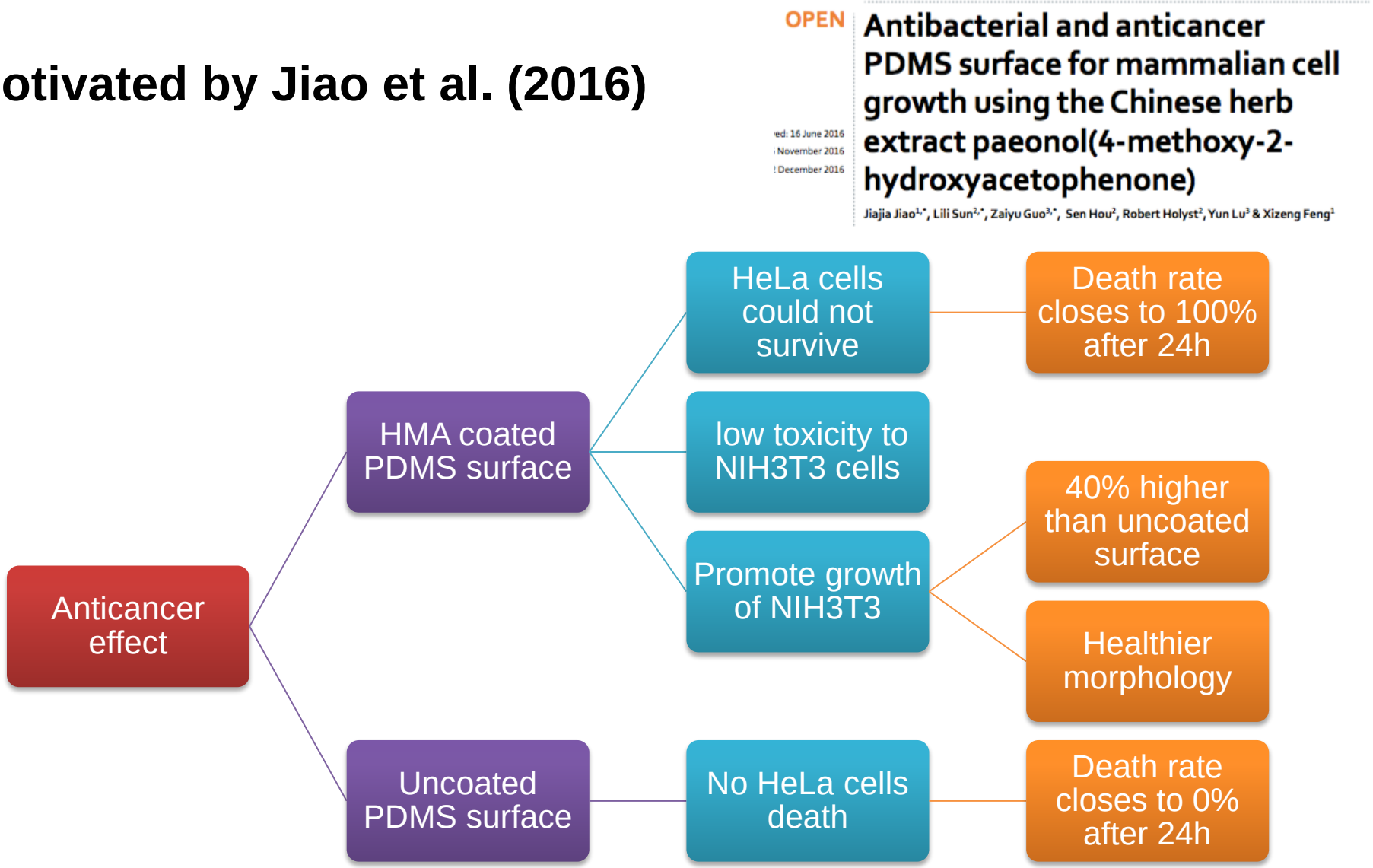
Gomes, M., Muratov, E., Pereira, M., Peixoto, J., Rosseto, L., Cravo, P., Andrade, C. and Neves, B., 2017. Chalcone derivatives: promising starting points for drug design. *Molecules*, *22*(8), pp.1210.

Targeted therapies v/s Dirty drug



Fojo, T., 2008. Commentary: novel therapies for cancer: why dirty might be better. *The oncologist*, *13*(3), p.277.
Pierce, G., 2012. Should we clean up the reputation of "dirty drugs"?. *Canadian Journal of Physiology and Pharmacology*. 90, 1333–1334.

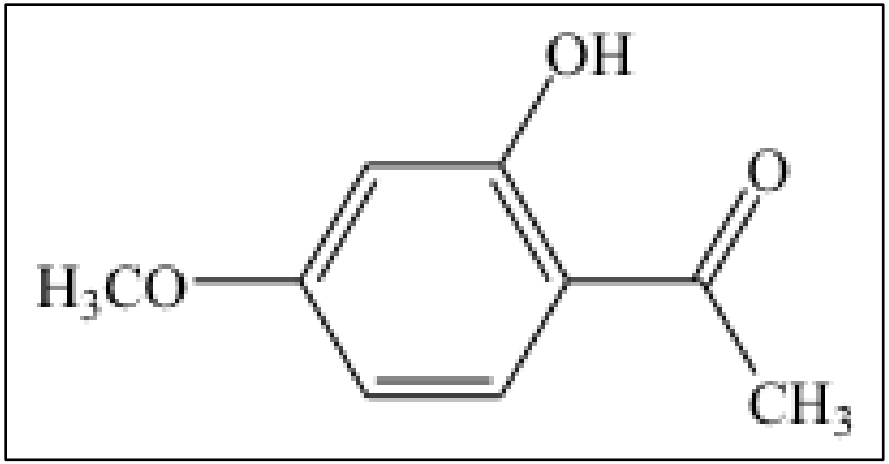
Motivated by Jiao et al. (2016)



Jiao, J., et al., 2016. Antibacterial and anticancer PDMS surface for mammalian cell growth using the Chinese herb extract paeonol (4-methoxy-2-hydroxyacetophenone). *Scientific reports*, 6, p.38973

2-Hydroxy-4-Methoxyacetophenone (HMA)

Also known as Paeonol, extracted from Chinese herb, *Paeonia moutan.* Traditionally use for anticancer and anti-inflammatory purposes

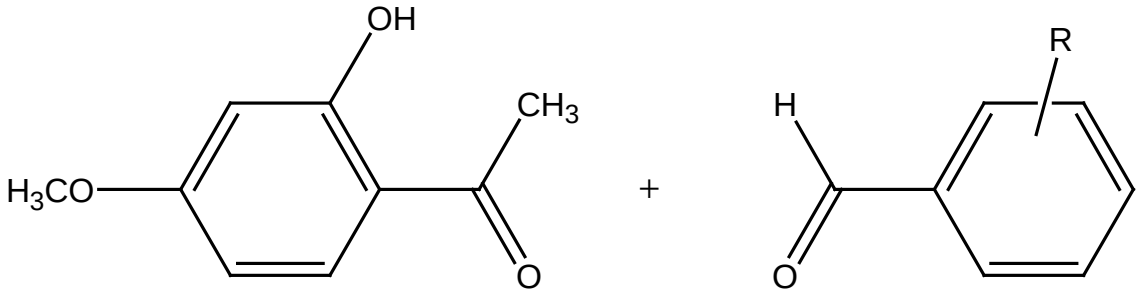


Anticancer effect:

- By inducing apoptosis (Ou et al., 2014)
- By reducing expression of mutant p53 as well as Bcl-2 in mice bearing EMT6 breast carcinoma (Tian et al., 2010; Wu et al., 2014).

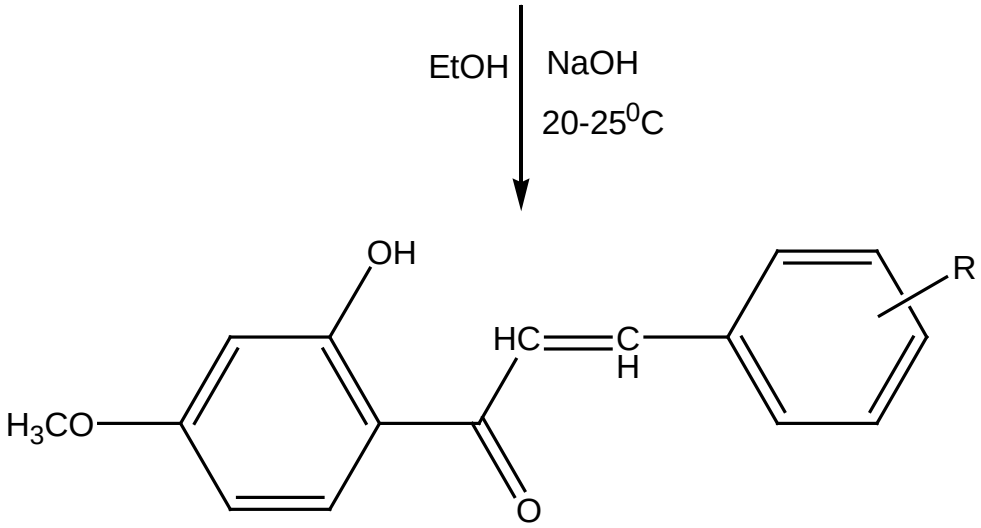
Ou, Y. et al., 2014. Antitumor and apoptosis induction effects of paeonol on mice bearing EMT6 breast carcinoma. *Biomolecules & therapeutics,* 22(4), p.341.
Tian, Y., et al., 2010. Antitumor effects of paeonol on mice bearing EMT6 breast infiltrating ductal carcinoma. *Latin American Journal of Pharmacy*, 5, p.369-375.
Wu, J., et al., 2014. Anti-tumor effect of Paeonol via regulating NF-κB, AKT and MAPKs activation: A quick review. *Biomedicine & Preventive Nutrition.*

Synthesis of HMA substituted chalcone derivatives



2-hydroxy-4-methoxy acetophenone

Substituted benzaldehyde

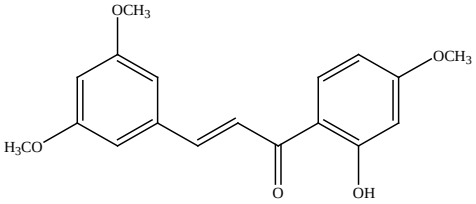
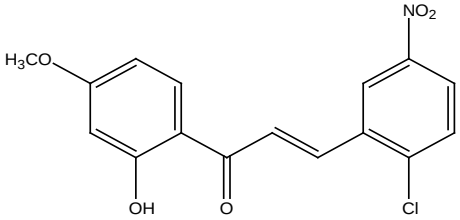
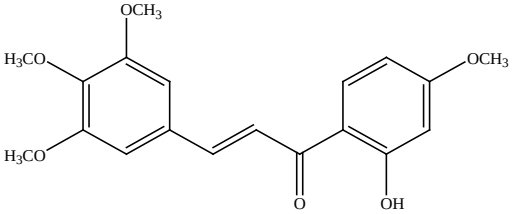


2-hydroxy-4-methoxy acetophenone substituted chalcones

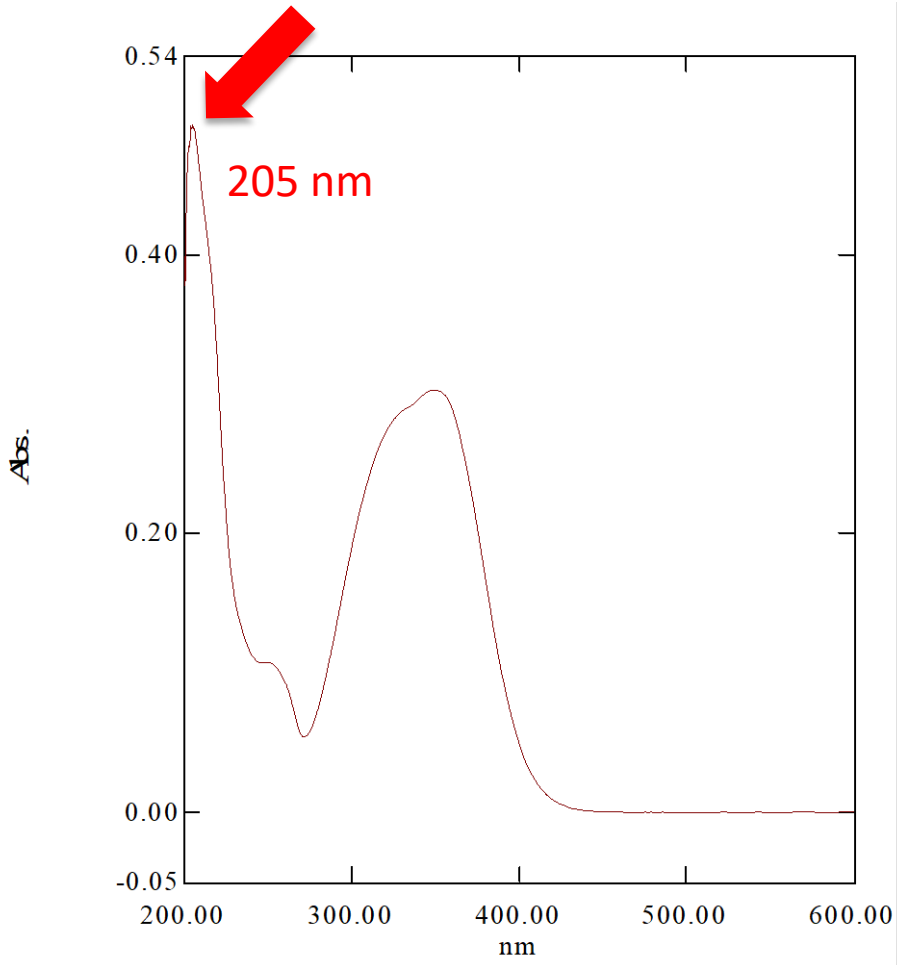
Results and Discussion

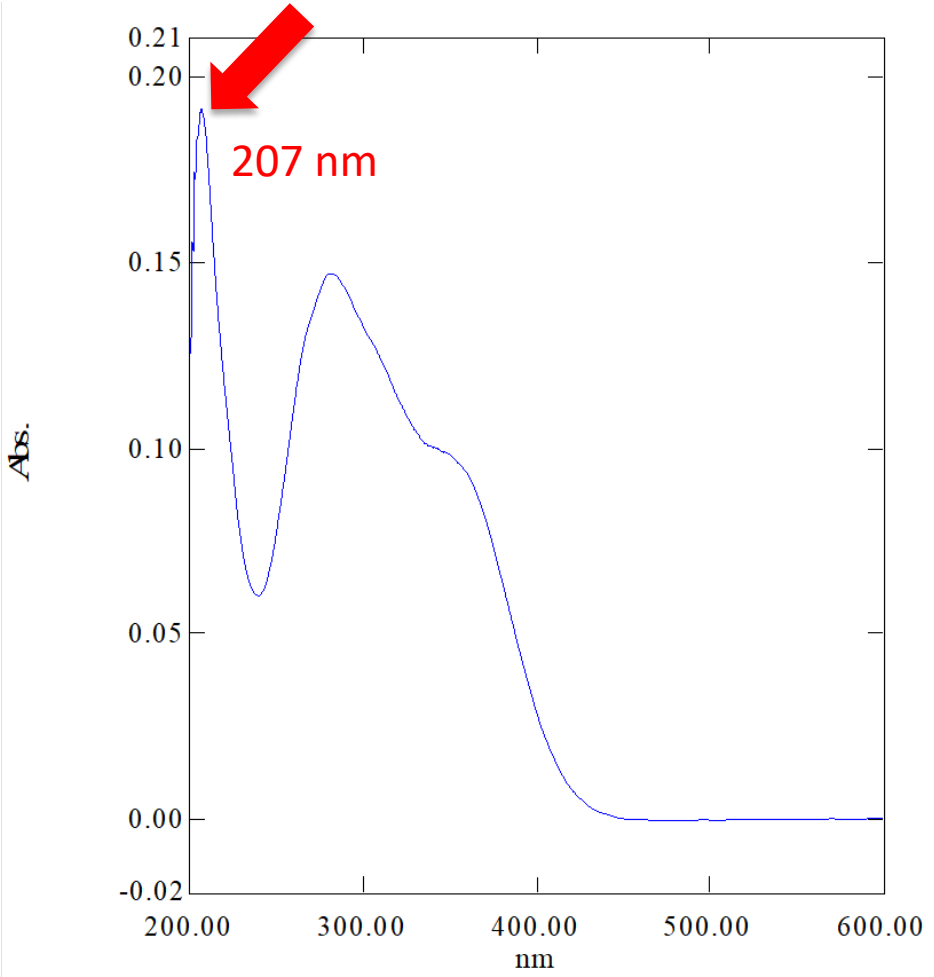
1. A total of ten HMA substituted chalcones were synthesised
2. All compounds were purified by column chromatography and chemical structures were charecterised by various spectroscopic analysis.
3. All compounds were screened for cell viability assay against selected cancer cell lines.
4. Out of 10 compounds,
    - ✓ 7 compounds were found active (IC_{50} < 20μM) against four different cancer cell lines
    - ✓ 3 compounds exhibited **potent activity** with IC_{50} values < 10μM against HT29, A549 and MCF-7 cancer cells

Physical Characteristics of potent compounds

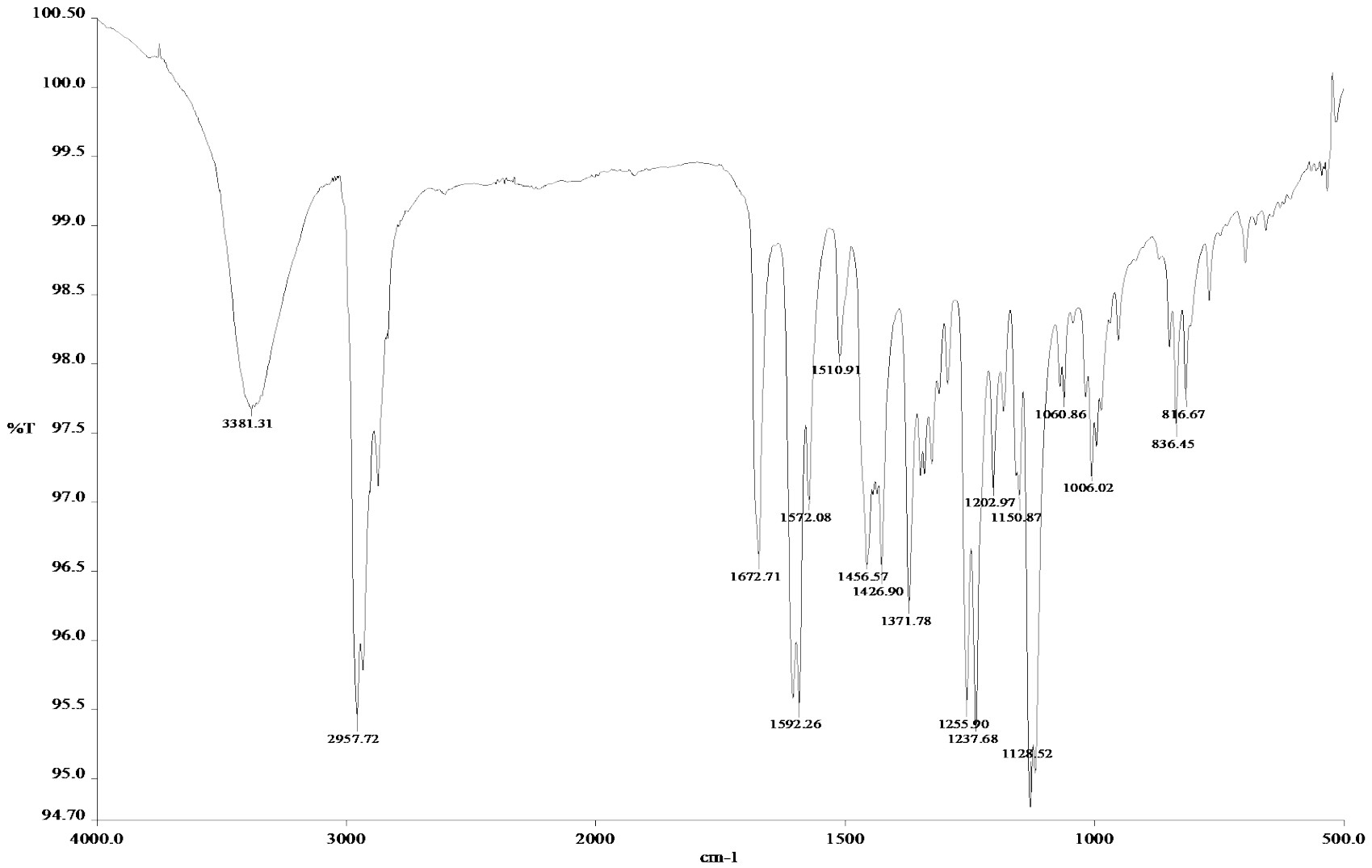
HMACs	Chemical Structure	Molecular weight	Yield (%)	Melting point (°C)	Rf value
LY-2		314.33	16.74	146.0 – 147.1	0.423
LY-8		333.72	3.66	193.4 – 193.9	0.466
LY-10		344.36	25.89	110.0 – 112.0	0.106

UV spectrum of compound LY-2 and LY-8





IR spectrum of compound LY-10



Results and Discussion

UV spectra characterization:

the peak absorption was due to the electron excitation from the carbon-carbon π-system to the antibonding orbital of the carbonyl group, indicating the $\pi \rightarrow \pi^*$ transition

The λ max was consistently found in the range between 205 to 207 nm

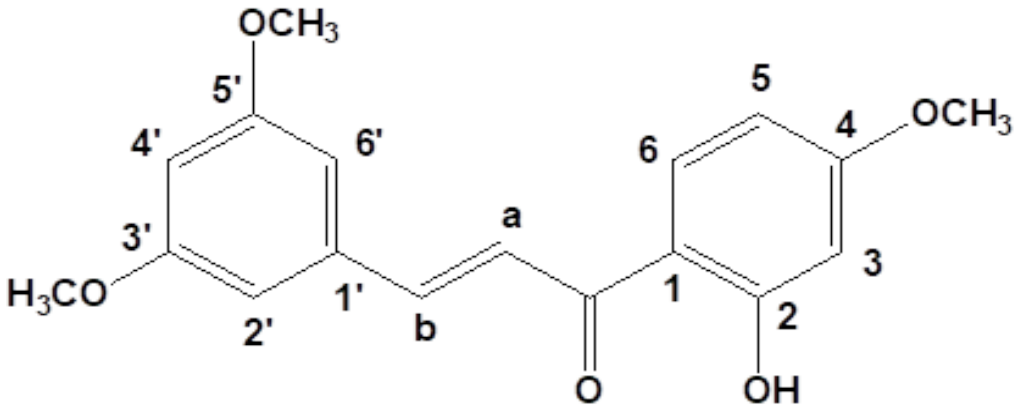
IR spectra characterization:

The C=O stretching was appeared at relatively lower wavenumber from 1675 to 1629 cm^{-1}

α, β-unsaturated C=C group in the synthesised compounds gave rise to absorption in 1620 to 1600 cm^{-1} with increased intensity due to adjacent polar carbonyl group.

Both C=O and C=C unsaturated groups favoured the delocalisation of π electrons and the bands shifted to lower wavenumber.

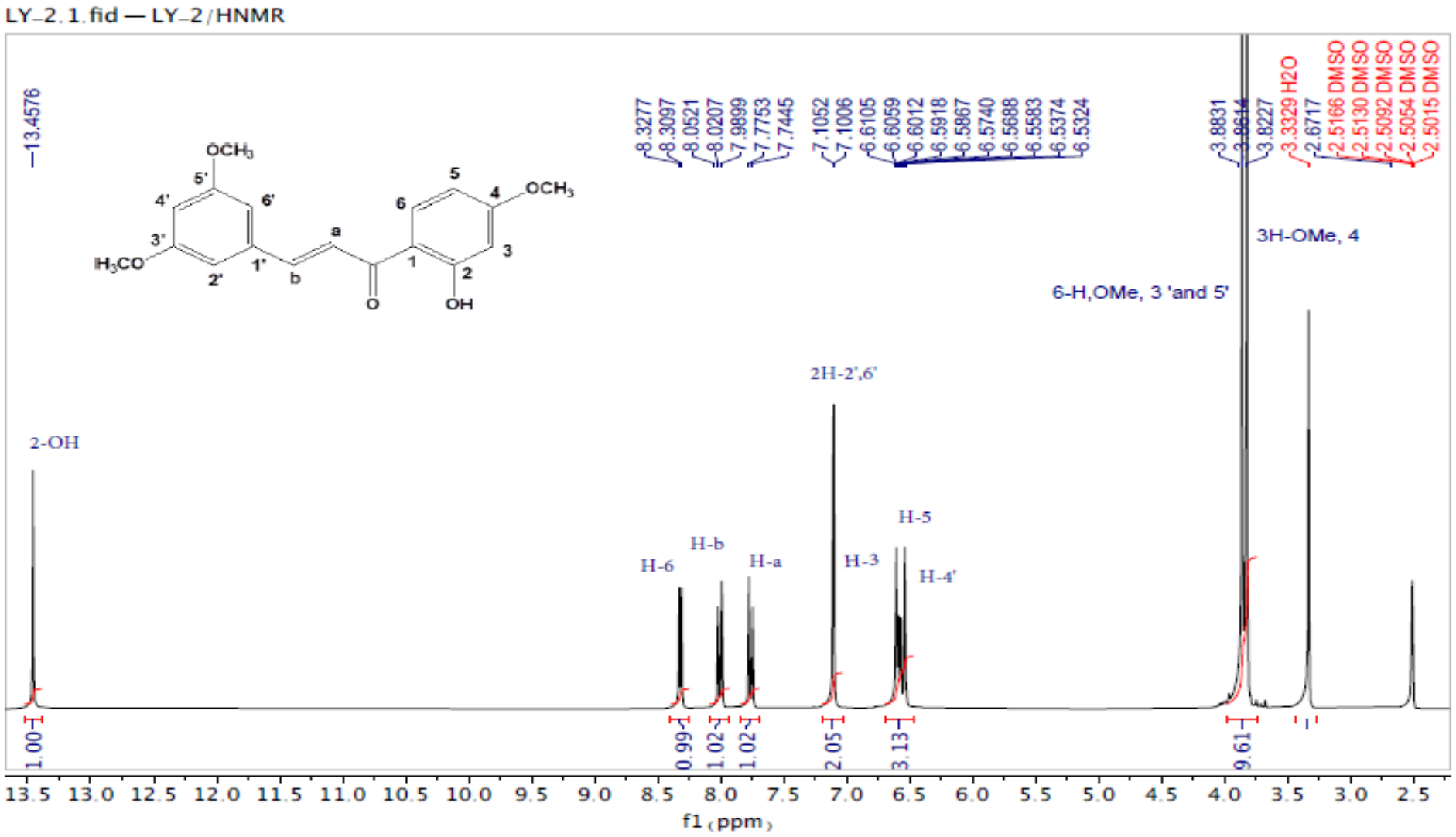
^{1}H-NMR and ^{13}-C- NMR of compound LY-2



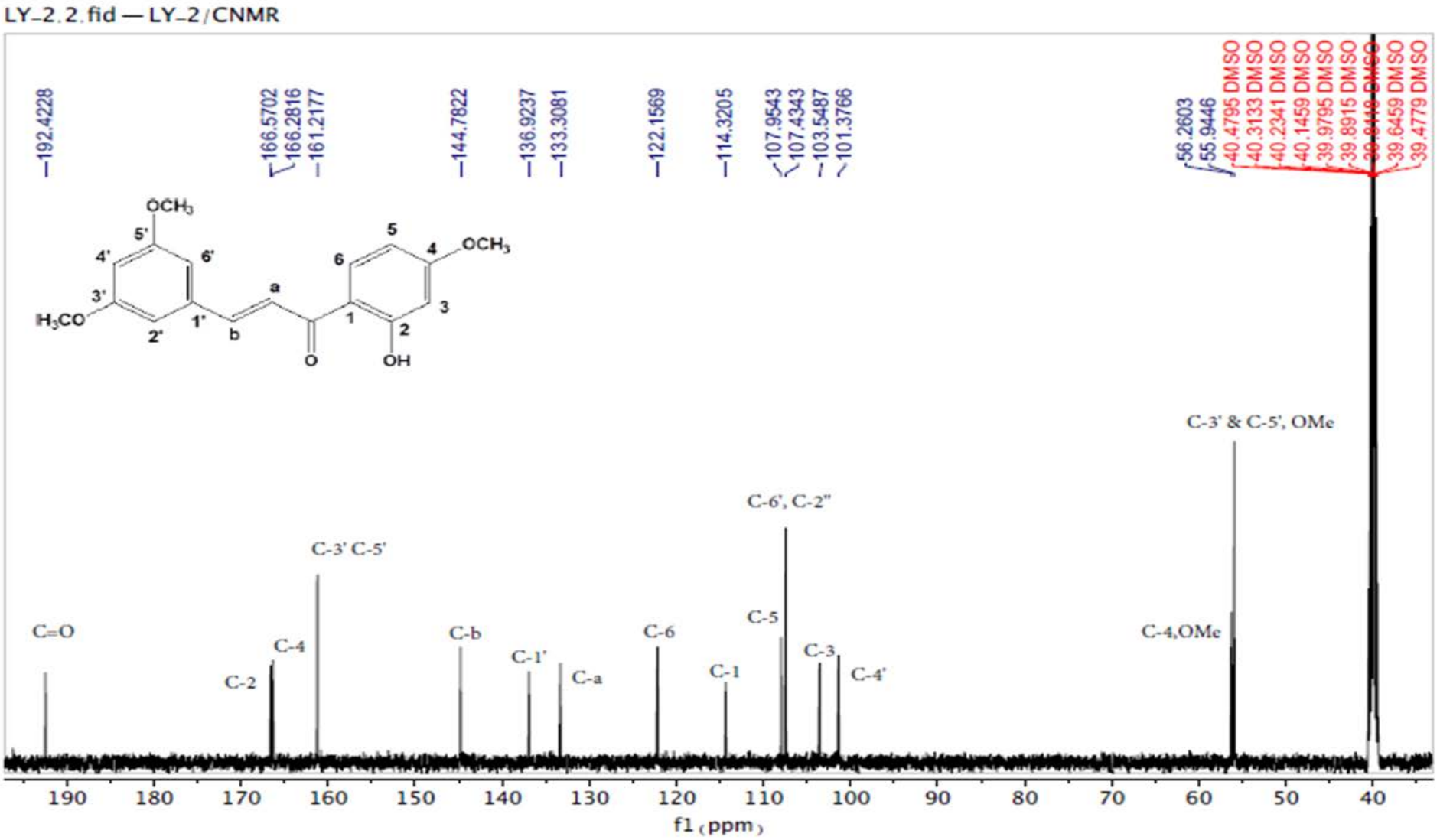
^{1}H-NMR (500 MHz, DMSO-*d6*) δ: 13.45 (s, OH-2) , 8.33-8.31 (d, *J*= 9Hz, H-6), 8.02-7.99 (d, *J*= 15.4 Hz, H-β), 7.77-7.74 (d, *J*= 15.4 Hz, H-α), 7.10 (s, 2H-2′ and 6′), 6.61 (s, H-3), 6.61-6.59 (dd, J= 2.35 Hz, 9.6 Hz, H-5), 6.57 (s, H-4′), 3.88 (s, 3H-OMe-4), and 3.82 (s, 6H-OMe-3′and 5′).

^{13}C-NMR (500MHz, DMSO-*d6*) δ: 192.42 (C=O), 166.57 (C-2),166.28 (C-4), 161.21 (C-3′,C-5′), 144.78 (C- β), 136.92 (C-1′), 133.30 (C- α), 122.15 (C-6), 114.32 (C-1), 107.95 (C-5), 107.43 (C-6′,C-2′), 103.54 (C-3), 101.37 (C-4′), 56.26 (C-4 OMe), and 55.94 (C- 3′, C-5′ OMe).

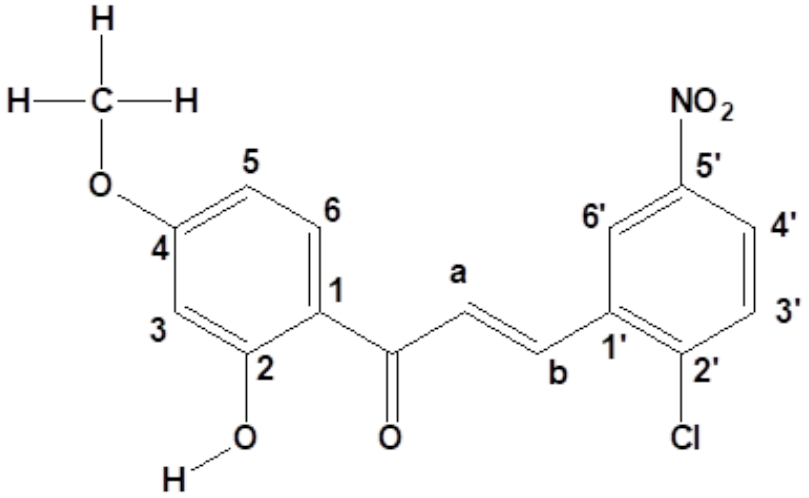
^{1}H-NMR of compound LY-2



^{13}C- NMR of compound LY-2



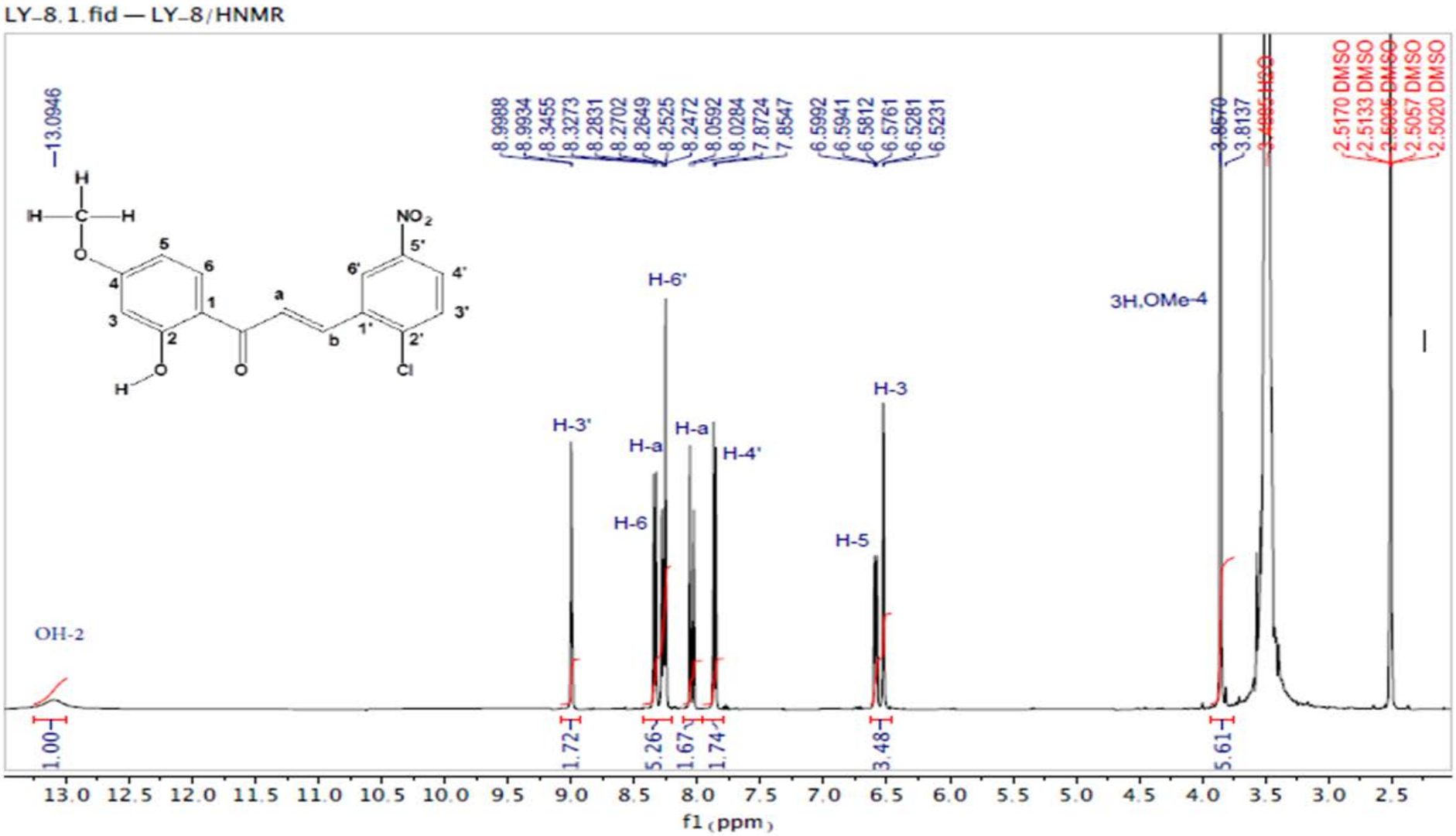
^{1}H-NMR and ^{13}-C- NMR of compound LY-8



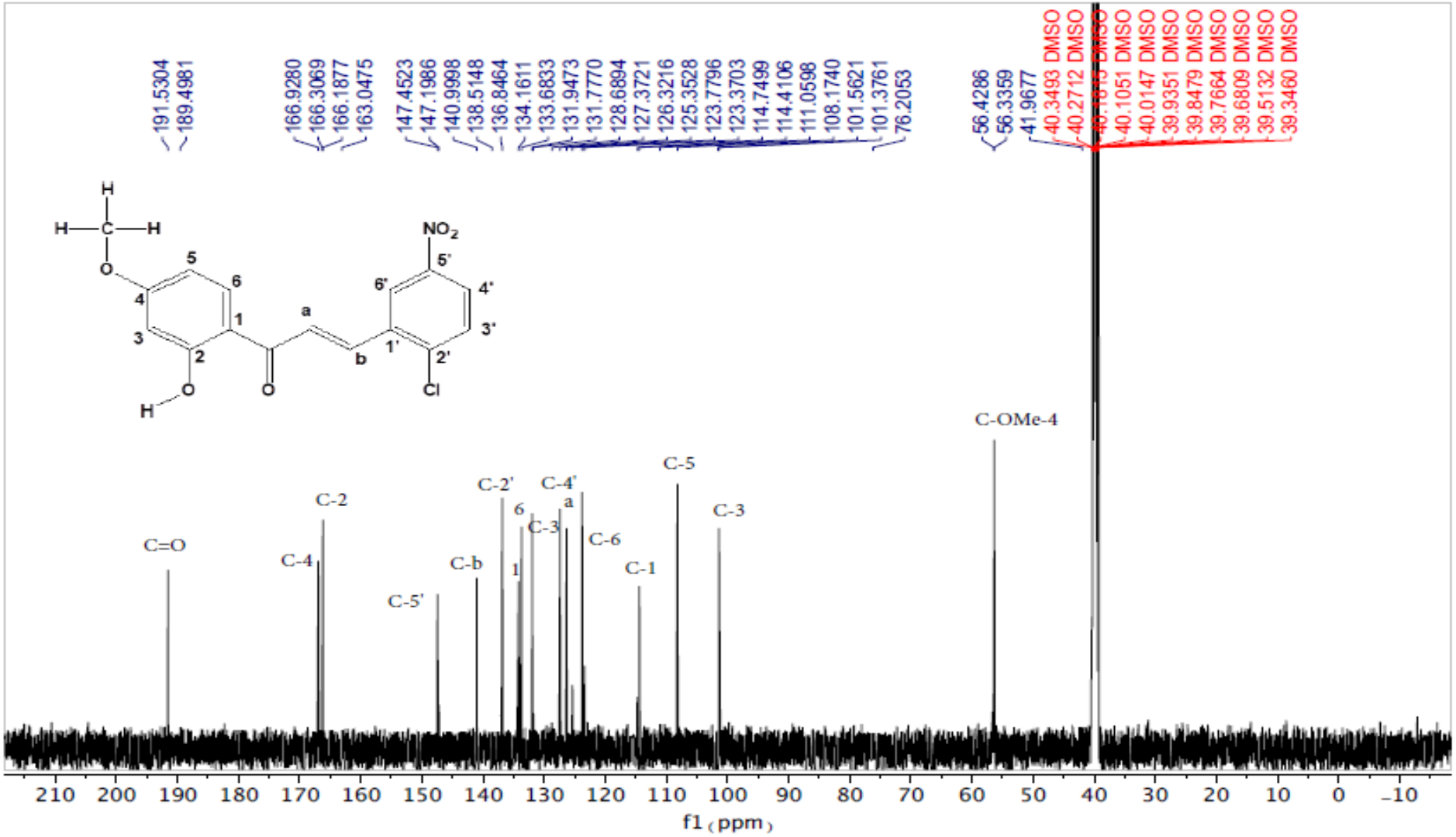
^{1}H-NMR (500 MHz, DMSO-*d6*) δ: 13.09 (s, OH-2) , 9.00-8.99(d, *J*= 2.7Hz, H-3′), 8.35-8.33 (d, *J*= 9.1 Hz, H-6), 8.28-8.25 (d, *J*= 15.3 Hz, H- β), 8.24 (s, H-6′), 8.06-8.03 (d, J= 15.4 Hz, H- α), 7.87-7.85 (d, J= 15.4 Hz, H-4′), 6.60-6.58 (dd, J= 2.55 Hz, 9 Hz, H-5), 6.52(s,H-3) and 3.85 (s, 3H-OMe-4).

^{13}C-NMR (500MHz, DMSO-*d6*) δ: 191.53 (C=O), 166.92(C-2),166.30 (C-4), 141.45(C-5′), 140.99(C- β), 136.85 (C-2′), 134.16(C-1′), 133.68 (C-6), 131.94 (C-3), 127.37(C-4′), 126.32(C- α),123.77 (C-6),114.41 (C-1), 108.17 (C-5), 101.37(C-3) and 56.33(C-4 OMe).

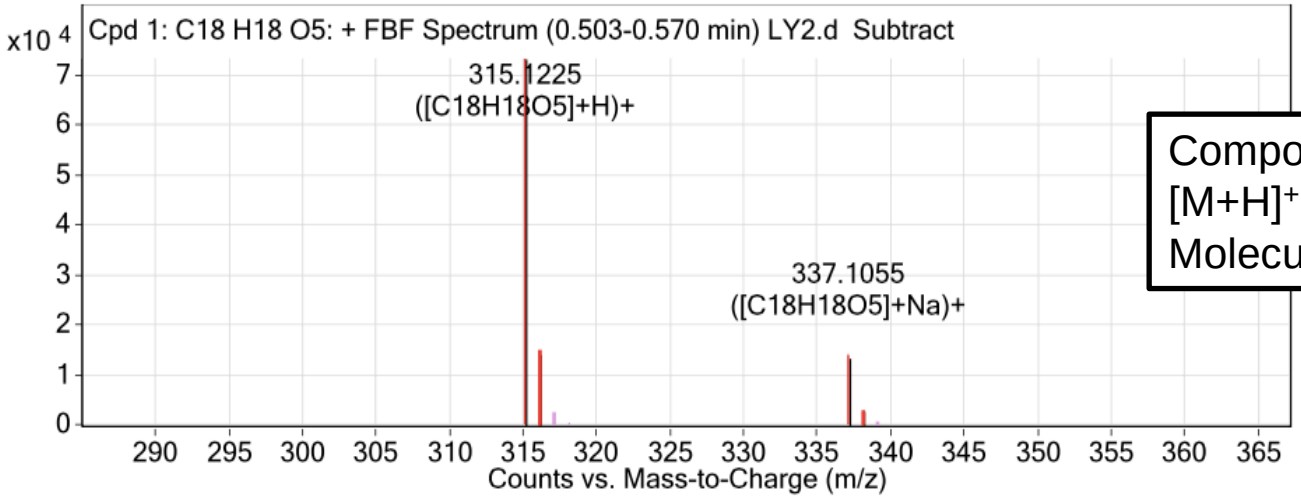
^{1}H-NMR sprectrum of compound LY-8



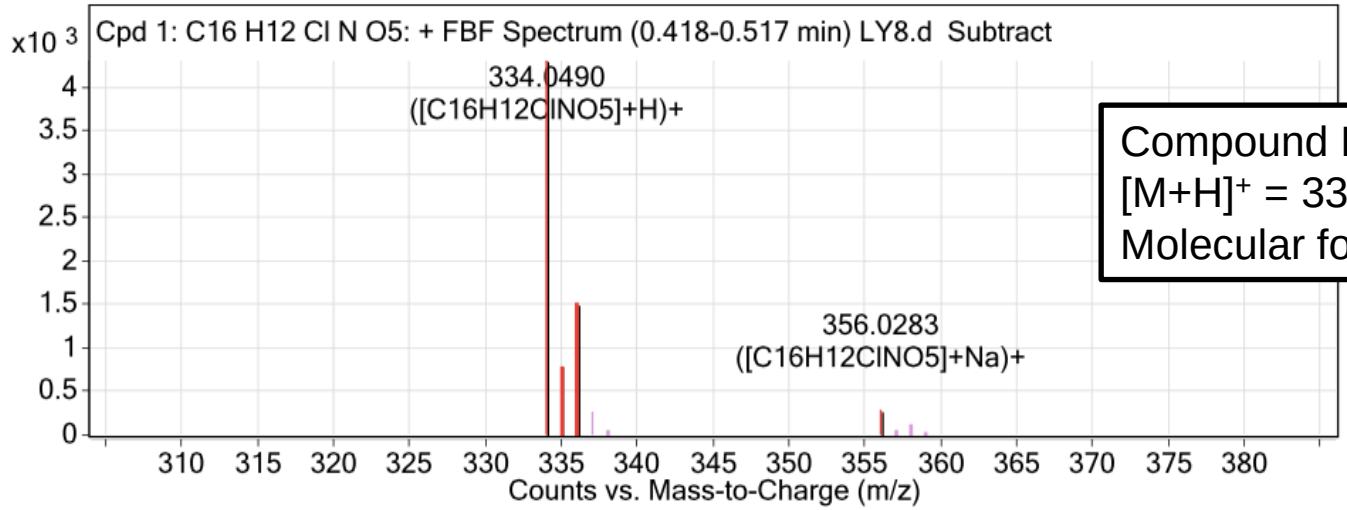
^{13}C- NMR of compound LY-8



Mass spectra of compounds LY-2 and LY-8

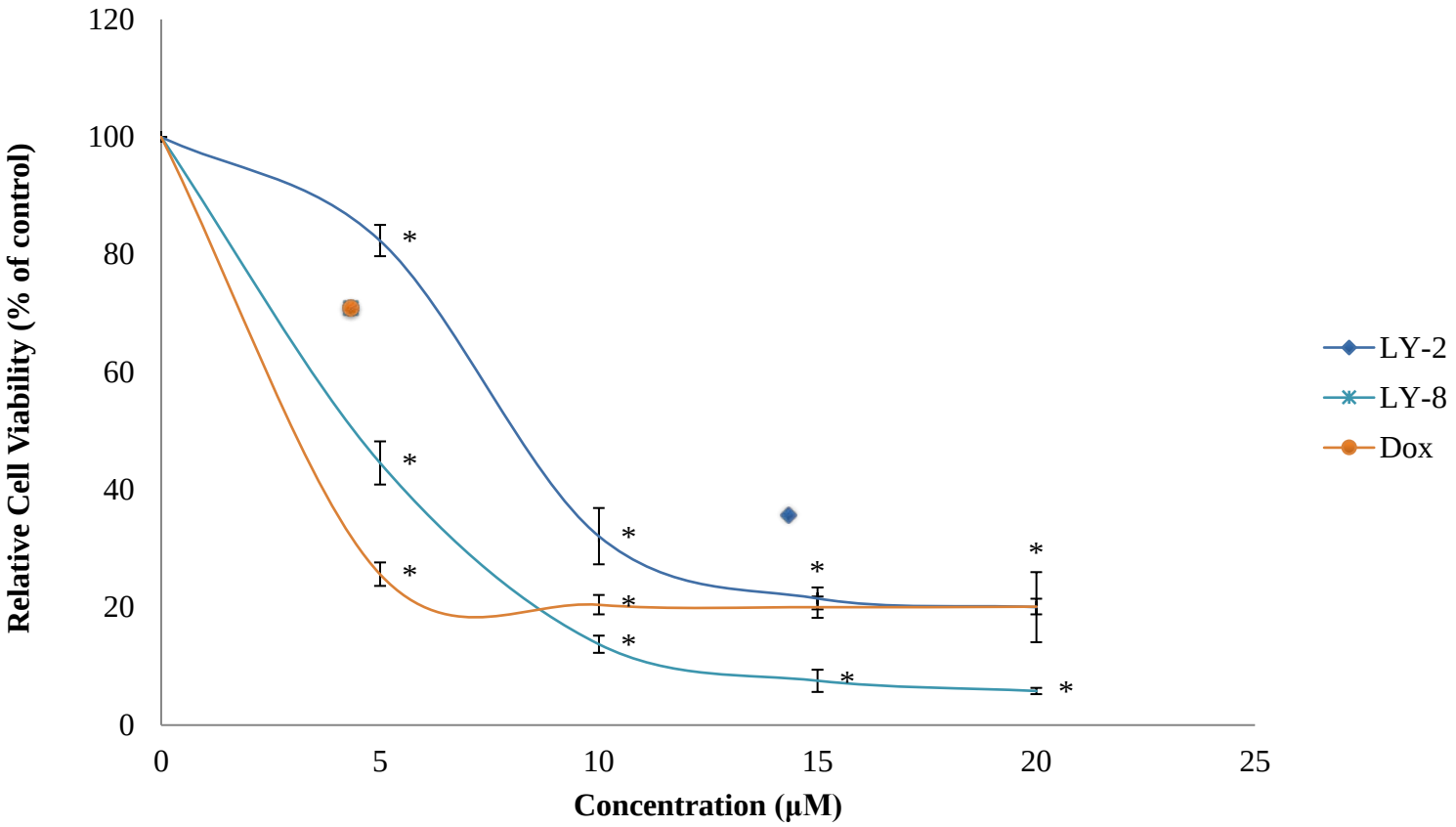


Compound LY-2
$[M+H]^+$ = 315.12 *m/z*
Molecular formula = $C_{18}H_{18}O_5$



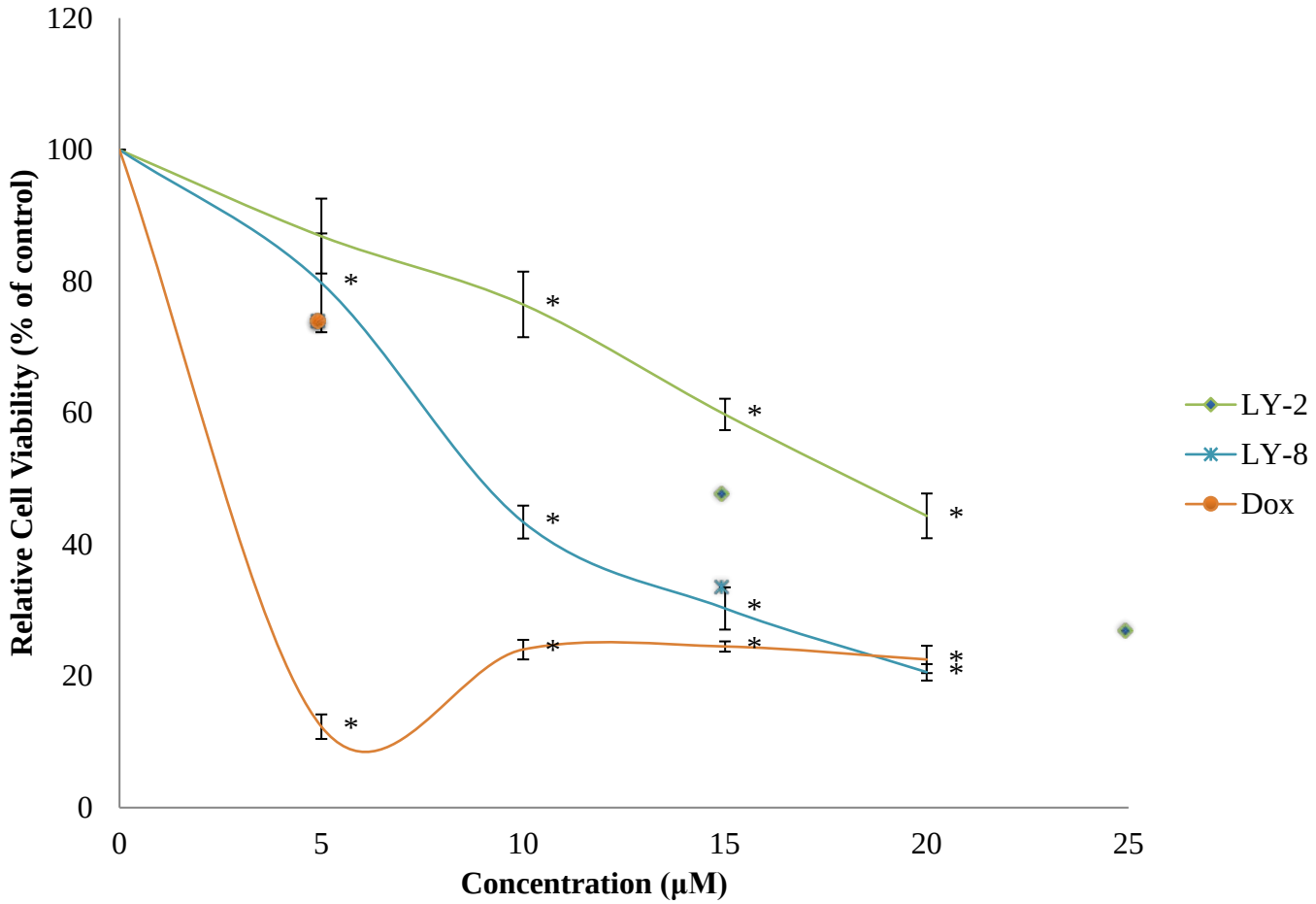
Compound LY-8
$[M+H]^+$ = 334.05 *m/z*
Molecular formula = $C_{16}H_{12}ClNO_5$

Human colorectal cancer cells (HT29)



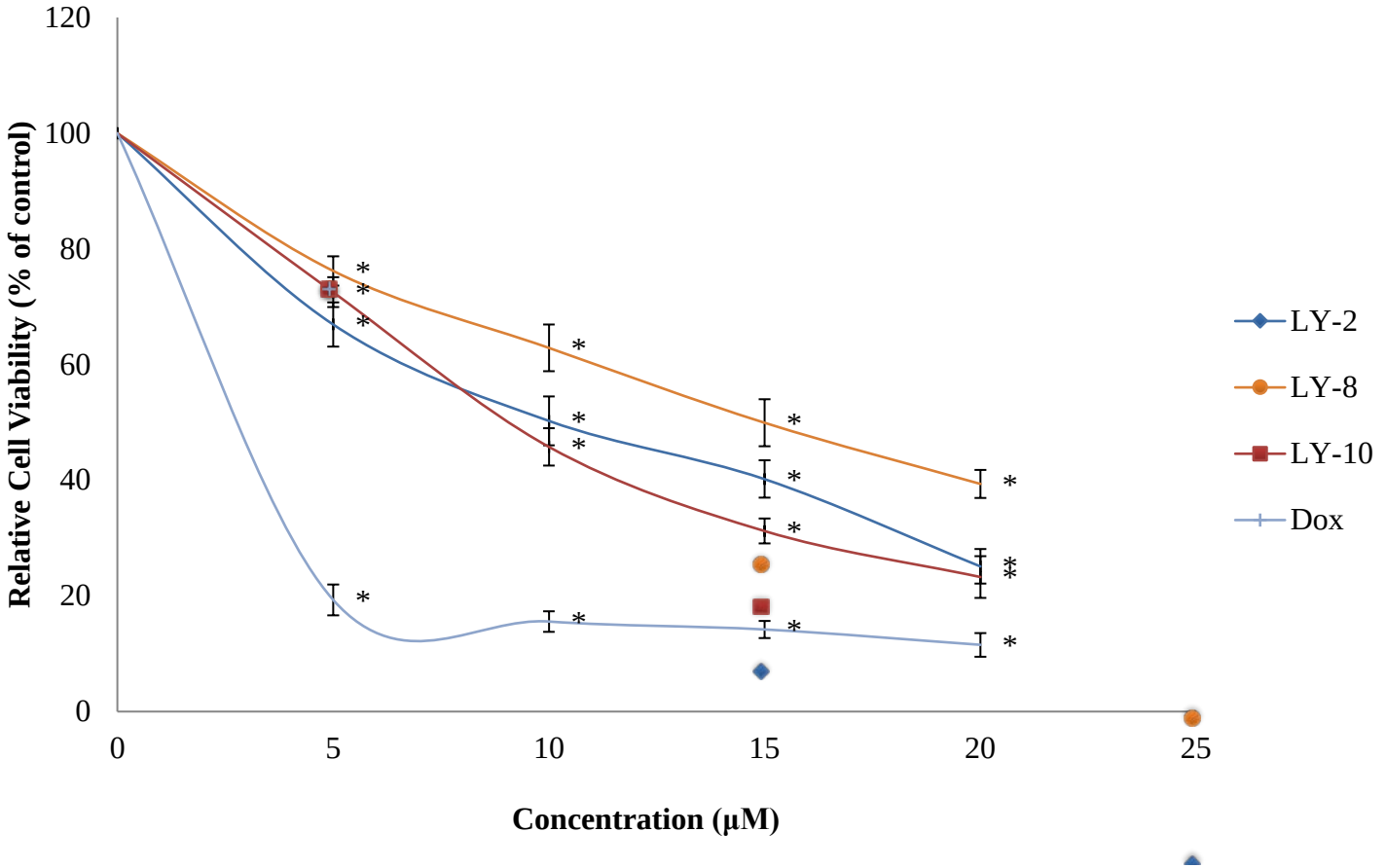
Relative cell viability (mean ± SD) of HT29 cells treated with a series of concentration of potent compounds for 48 hours. *P*<0.05 (*) (One-way ANOVA)

Human lung cancer cells (A549)



Relative cell viability (mean ± SD) of A549 cells treated with a series of concentration of potent compounds for 48 hours. *P*<0.05 (*) (One-way ANOVA)

Human breast cancer cells (MCF-7)



Relative cell viability (mean ± SD) of MCF-7 cells treated with a series of concentration of potent compounds for 48 hours. *P*<0.05 (*) (One-way ANOVA)

Results and Discussion

The α, β-unsaturated ketone unit remained critical for the anticancer activity of potent compounds.

Structure-activity relationship:

Electron-withdrawing group

LY-8 bearing 2′-chloro and 5′-nitro groups

- Further enhance the electron deficiency of the β carbon and carbonyl carbon
- A markedly different degree of sensitivity observed in HT29 and A549 cancer cells.
- Thus, greater inhibitory activity

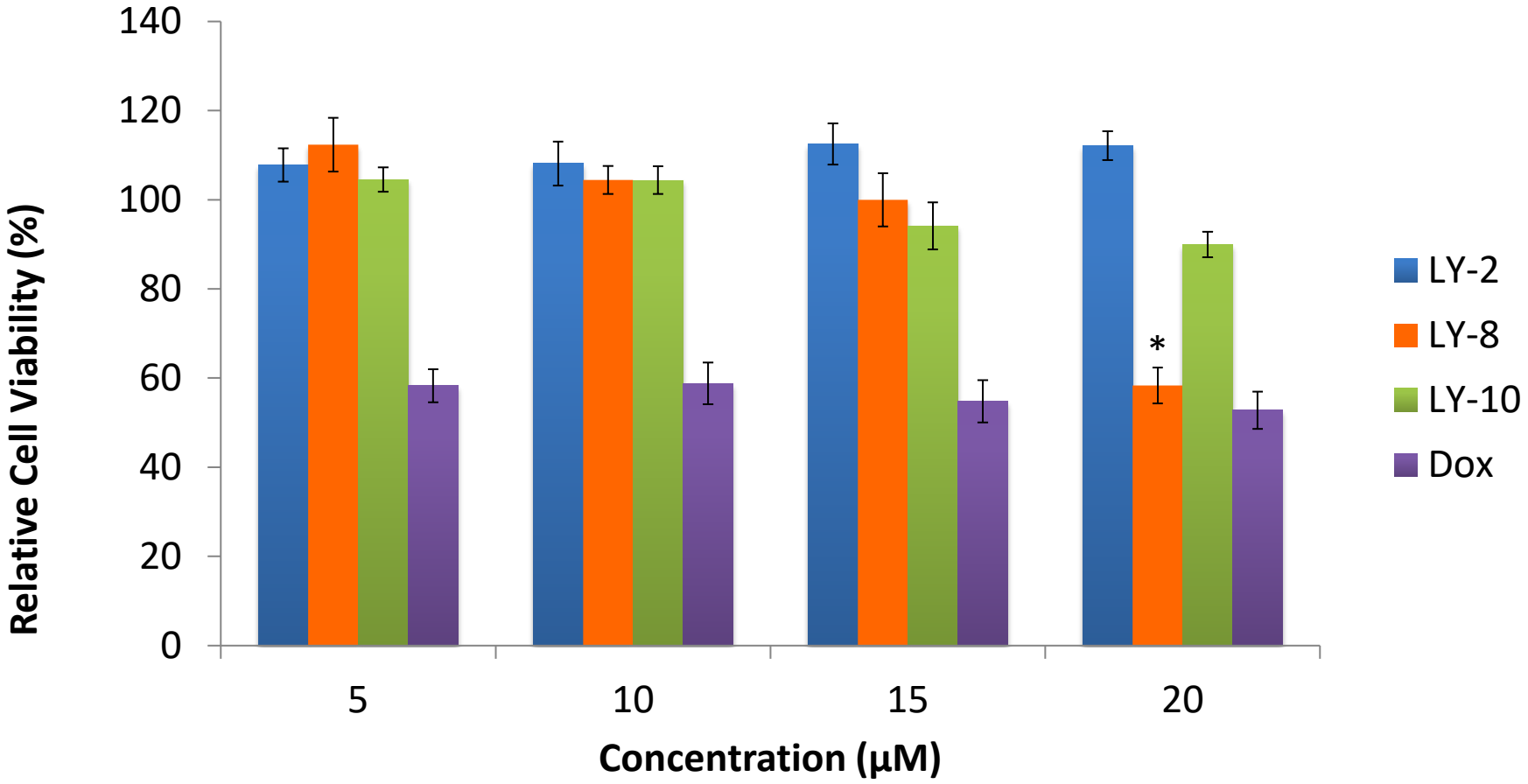
Electron-donating group

LY-2 with 3′,5′-dimethoxy substitution

LY-10 with 3′,4′,5′-trimethoxy substitution

- Selectively active on MCF-7 breast cancer cells
- Methoxy group at 3rd position on phenyl ring B played an important role in cytotoxicity

Non-cancerous Human Dermal Fibroblast (HDF)



Relative cell viability (mean ± SD) of HDF cells treated with a series of concentration of potent compounds for 48 hours. *P*<0.05 (*) (One-way ANOVA)

The degree of selectivity of 3 potent compounds

IC_{50} represents the strength and sensitivity of the drug on the cells, the “more anticancer activity” implies “more toxicity” on non-cancerous cells.

- Three potent compounds showed IC_{50} values > 20 μM on HDF cells, demonstrating favorable safety profile

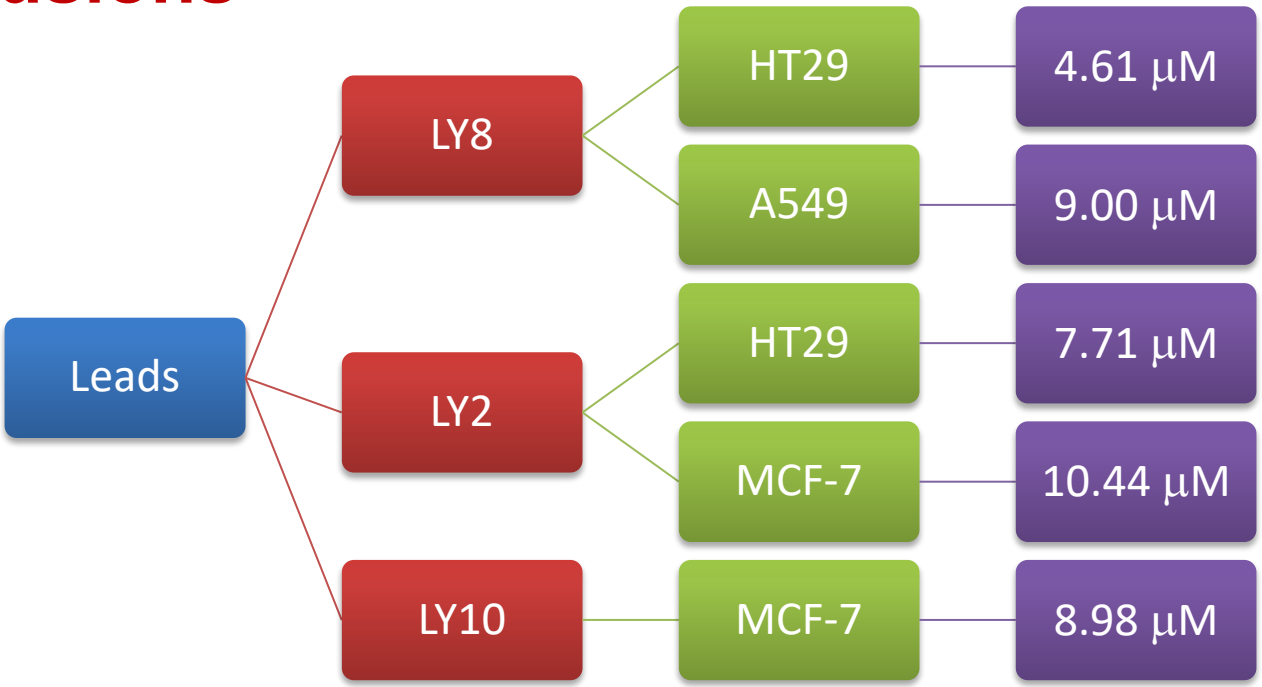
Selectivity index (SI) was reported to determine the ability of synthesised compounds to discriminate between non-cancerous and cancerous cells

- LY-8 exhibited the highest selectivity against HT29 colorectal cancer cells, showing SI value of >4.34, which was about two times more selective than doxorubicin (SI value 2.4).
- LY-10 also showed SI value >2.23 against MCF-7 breast cancer cells which also higher than that of doxorubicin (SI value 2.1)

IC_{50} values and Selective Index (SI)

Cell line	IC_{50} of compounds (μM)			
	LY-2	LY-8	LY-10	Doxorubicin
MCF-7	10.44 ± 1.52	-	8.98 ± 0.79	0.62 ± 0.14
HT29	7.72 ± 0.59	4.61 ± 0.29	-	0.52 ± 0.12
A549	-	9.00 ± 0.82	-	0.34 ± 0.02
HDF	>20	>20	>20	1.2 ± 0.11
SI HDF/MCF-7	> 1.92	-	> 2.23	2.1
SI HDF/HT29	> 2.59	> 4. 34	-	2.4
SI HDF/A549	-	> 2.22	-	3.7

Conclusions



- ✓ These compounds showed only minimal inhibition effect on non-cancerous HDF cells with IC_{50} > 20 µM.
- ✓ Compounds LY-8 and LY-2 are specifically fitted as dirty drug candidates due to their broad and significant anticancer activity.
- ✓ The active compounds from current study could be considered as lead molecules for further studies such structure optimization, SAR, molecular docking studies, *in vivo* Pharmacokinetics, bioavailabity and toxicity studies to develop as anticancer drug.

Acknowledgments

I would like to express my sincere gratitude to my research supervisor, Dr. Naveen Kumar Hawala Shivashekaregowda for his kind encouragement and knowledgeable guidance throughout the course of the project. I am extending my heartfelt thanks to my co-supervisors, Dr. Foo Jhi Biau and Dr. Nusaibah binti Abdul Rahim who put their utmost efforts to arrange necessary materials and facilities, also provided helpful feedbacks and comments at various stages of my research. I also gratefully acknowledge the funding source, Taylor's Research Grant Scheme: TRGS/MFS/1/2016/SOP/009 for consumables support.

I would specially thank Dr. Syed Adnan Ali Shah for conducting the NMR study of synthesised compounds and Loh Jian Sheng for providing me with his assistance in cell viability assay.