Indiana University Kokomo

Design and Synthesis of Cytotoxic Compounds by Targeting Cancer Metabolism

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An Overview of the Presentation

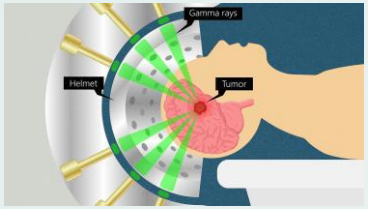
1. Importance of designing new cytotoxic compounds for the treatment of Cancer.
2. Design of compounds based on cancer metabolism.
3. Anticancer properties of three new series of compounds which were designed and synthesized in my lab.

What is Cancer?

- Cancer is a group of more than 100 different diseases. It can develop almost anywhere in the body.
- Normal cells grow and divide to make new cells as the body needs them. Usually, cells die when they get too old or damaged. Then, new cells take their place.
- Abnormal and uncontrolled cell growth is characterized as cancer cells. These cells may form a mass called a tumor.

Treatment of Cancer

01



Radiation Therapy

02

Surgery

03

Chemotherapy

Besides surgery and radiation therapy, chemotherapy still remains the most common approach in cancer treatment.

Challenges to the Success of Chemotherapy

01

Tumour-selectivity

Lack of tumour-selectivity is a key factor that pose serious challenges to the success of chemotherapy

02

Multidrug Resistance (MDR)

Multidrug resistance, by which many cancers develop resistance to chemotherapy drugs, is a key factor in the failure of many forms of chemotherapy.

03

Side Effects

Fatigue, Hair loss, Easy bruising and bleeding, Infection, Anemia (low red blood cell counts), Nausea and vomiting, Appetite changes, Constipation, etc

Development of Novel Drug Candidates

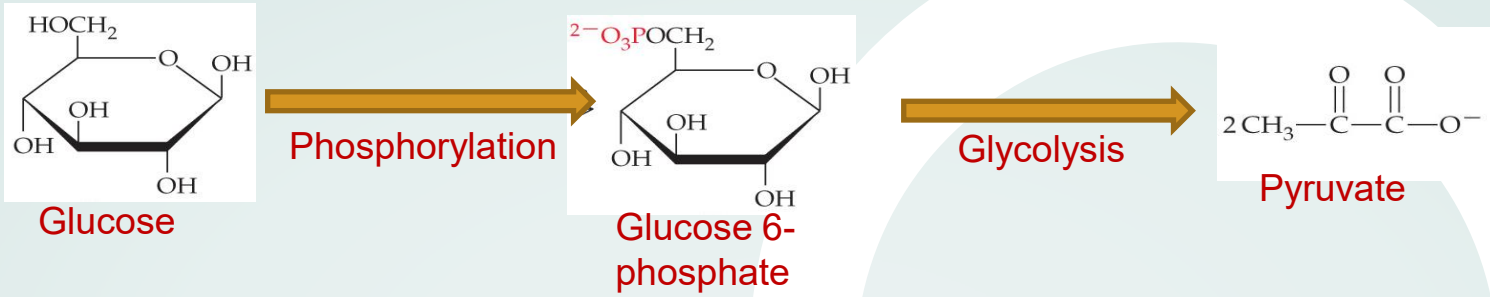
- There is a need for novel drug candidates that can selectively target tumors compared to normal cells and to treat MDR tumors.
- Therefore, many studies have been published for developing novel cytotoxic compounds of natural, semisynthetic, and synthetic origins.
- Many of these cytotoxic compounds were designed:
  - by targeting cancer metabolism
  - based on a multi-targeting action mechanism

Metabolism in Cancer Cells

Cancer cells show different metabolisms as compared to normal cells to meet their resources and energy needs.

- Cancer cells exhibit a high rate of anabolic metabolism, thereby, take up large amounts of glucose and glutamine to fuel:
  - citric acid or TCA cycle and oxidative phosphorylation (OXPHOS)
  - pentose phosphate pathway for nucleotide synthesis
  - amino acid synthesis for proteins
  - lipid synthesis

Glucose Metabolism in Normal Cells



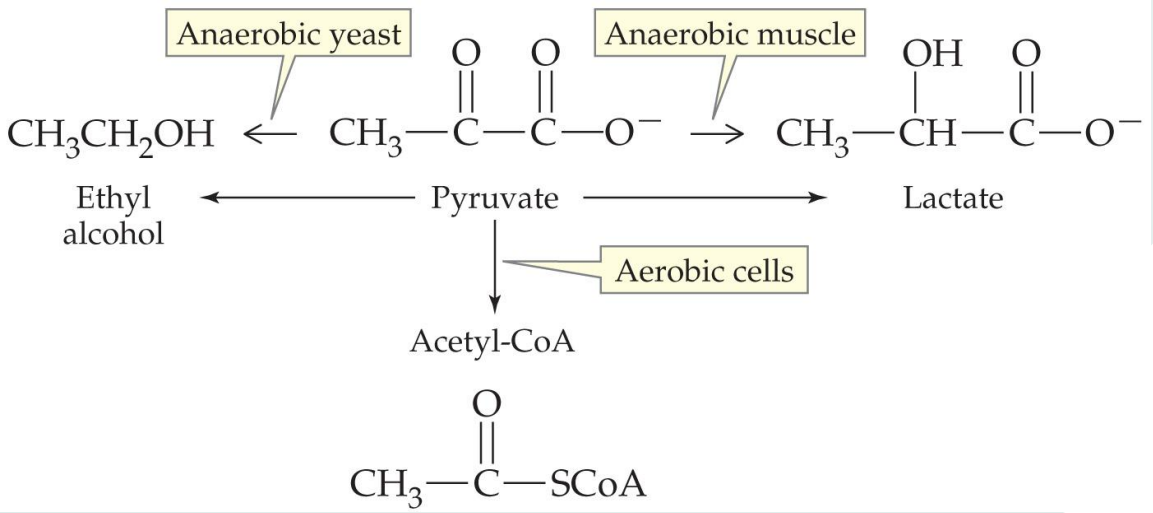
- Glucose is metabolized to pyruvate in a series of 10 enzyme-catalyzed reactions known as **glycolysis**. The net result is:
  - Conversion of glucose to two pyruvate molecules.
  - Net production of two ATP molecules.
  - Production of two molecules of reduced coenzyme NADH from NAD^+.

Net result of glycolysis

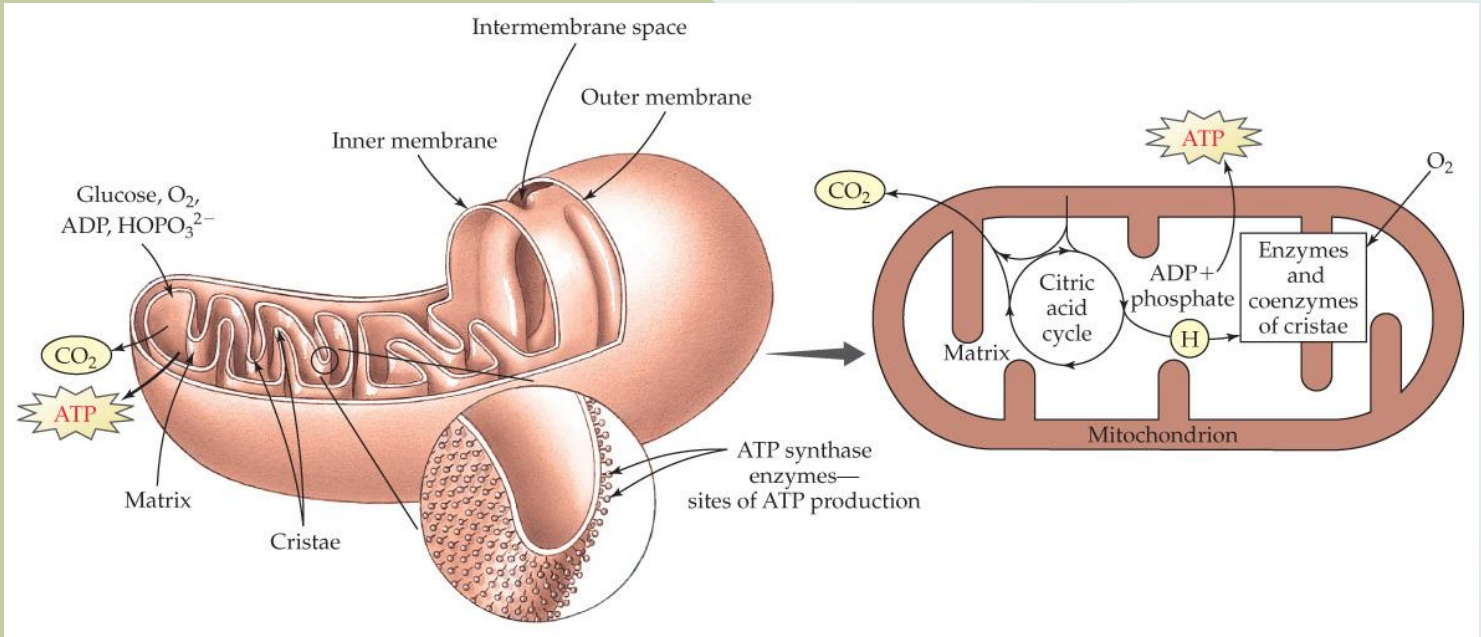
$$\underset{\text{Glucose}}{C_6H_{12}O_6} + 2\,NAD^+ + 2\,HOPO_3^{2-} + 2\,ADP \longrightarrow 2\,\underset{\text{Pyruvate}}{CH_3{-}C(=O){-}C(=O){-}O^-} + 2\,NADH + 2\,ATP + 2\,H_2O + 2\,H^+$$

The Fate of Pyruvate in Normal Cells

- Under aerobic conditions, pyruvate is converted to acetyl-CoA.
- Under anaerobic conditions, pyruvate is instead reduced to lactate.
- Yeast converts pyruvate to ethanol under anaerobic conditions.



Mitochondria: The Cell's Powerhouse



Mitochondria, the cell's "power plants," are the most important of the organelles for energy production.

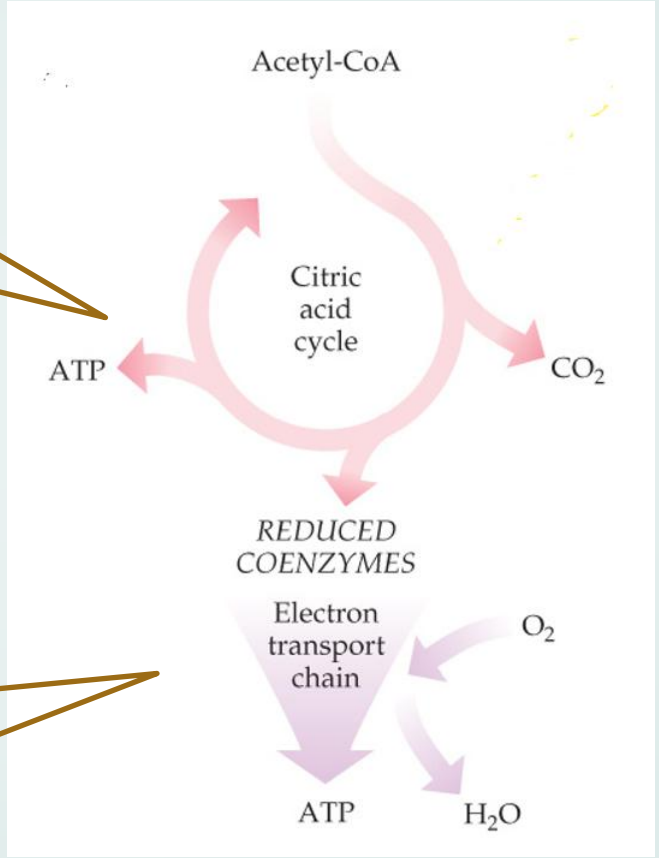
ATP Synthesis via Oxidative phosphorylation

Citric acid cycle

Acetyl-CoA is oxidized inside mitochondria by the citric acid cycle to yield CO_2 and reduced coenzymes

ATP Production

The energy transferred to the reduced coenzymes in the citric acid cycle is used to make ATP by the electron transport and oxidative phosphorylation

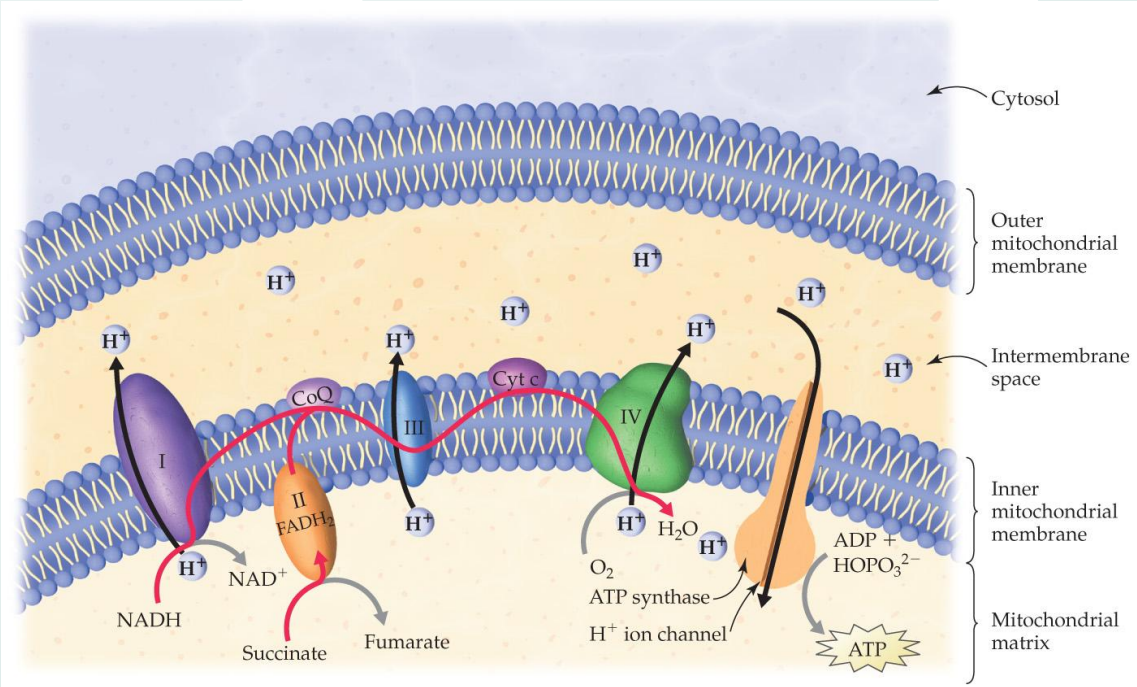


The Electron-Transport Chain and ATP Production

- The sequence of reactions is known as the electron-transport chain (also the respiratory chain).
- The enzymes and coenzymes of the chain are embedded in the inner membrane of the mitochondrion.

$$ADP + HOPO_3^{2-} + \Delta E \rightarrow ATP$$

$$2H^+ + 1/2\ O_2 + 2e^- \rightarrow H_2O$$



Back to the Cancer Cell's Metabolism

- For anabolic needs cancer cells are more dependent on mitochondrial function than normal cells.
- Therefore, glucose oxidation via mitochondrial pathway is essential for the generation of building blocks required for cancer cell proliferation.
- As a result, respiration is increased in cancer cells as compared to normal cells. Thus, consumption of O_2 is increased by:

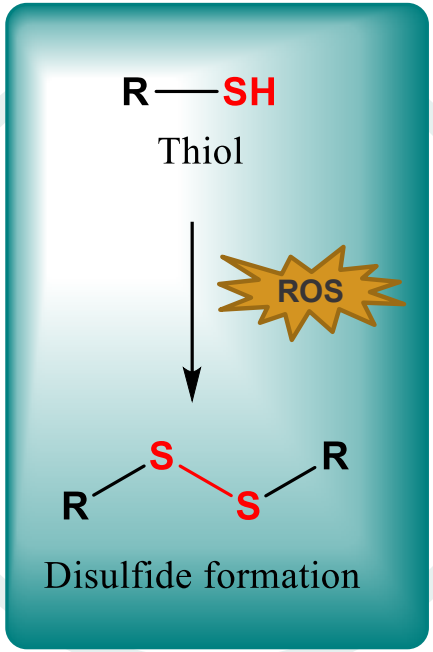
$$2\,H^+ + \tfrac{1}{2}\,O_2 + 2e^- \longrightarrow H_2O + \text{ROS}$$

ROS = Reactive Oxygen Species

$\cdot O_2^-$	O_2^{2-}	$HO\cdot$
Superoxide anion	Peroxide ion	Hydroxyl radical

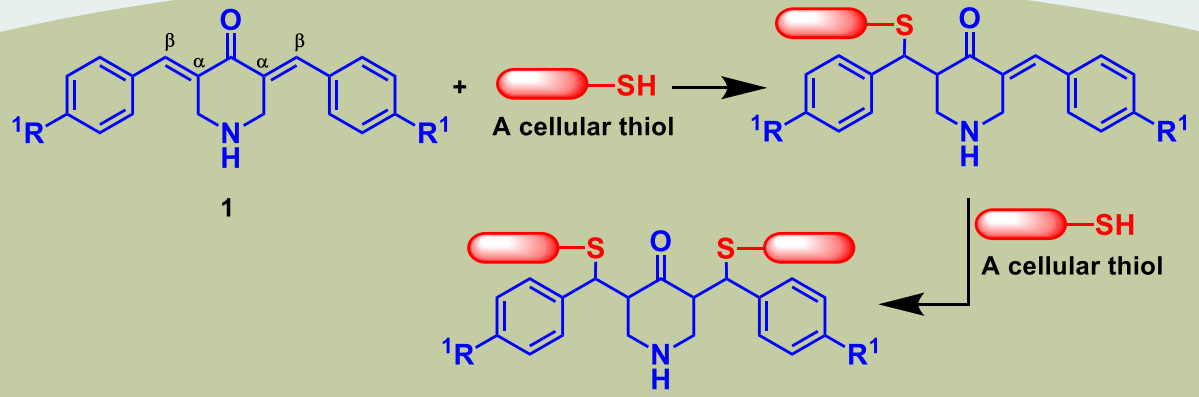
Controlling the effect of ROS

- ROS are very toxic that can kill cells.
- Therefore, cells have mechanisms to control ROS level.
- Normally, they use thiol macromolecules such as glutathione, thioredoxin (Trx) and glutathione S-transferases to control ROS.



Anticancer Drugs as Thiol Alkylators*

- The expression of thiol macromolecules is found to be elevated in cancers compared to normal tissues.
- Therefore, chemical agents that target these cellular thiols are likely to cause more harmful effects to tumor cells compared to normal cells.
- This is our potential strategy to develop tumor selective cytotoxins.



The sequential alkylations of cellular thiols by 3,5-bis(arylidene)-4-piperidones leading to the cell death of cancer cells.

*M. Hossain et al., *European Journal of Medicinal Chemistry* 2019, 183, 111687
*M. Hossain et al., *Current Medicinal Chemistry* 2021, 28, 2453-2464

Benzylidene cinnamoylhydrazides as cytotoxic agents*

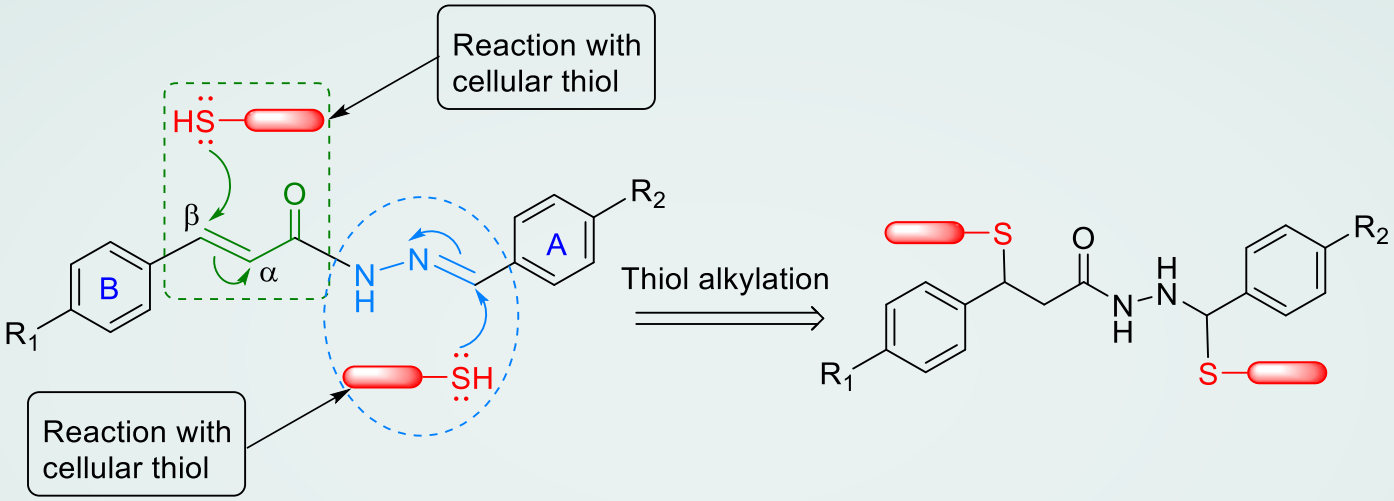


Figure: Possible modes of action of a representative compound with cellular constituents.

*M. Hossain et al., *Scientia Pharmaceutica* **2026**, 94, 86

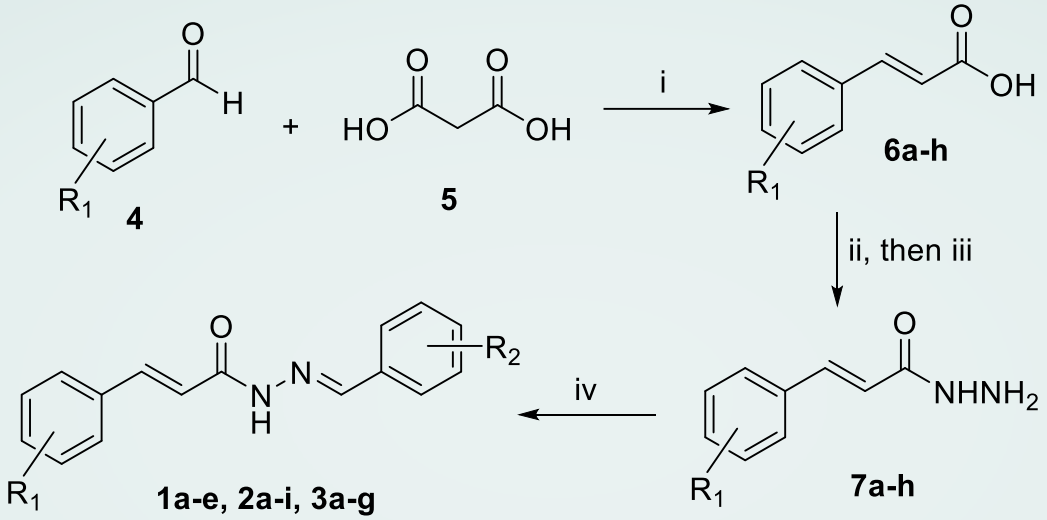
Acknowledgement to Students

Stephen Markley

Jacquelynn Wiles

Jolie Dionne

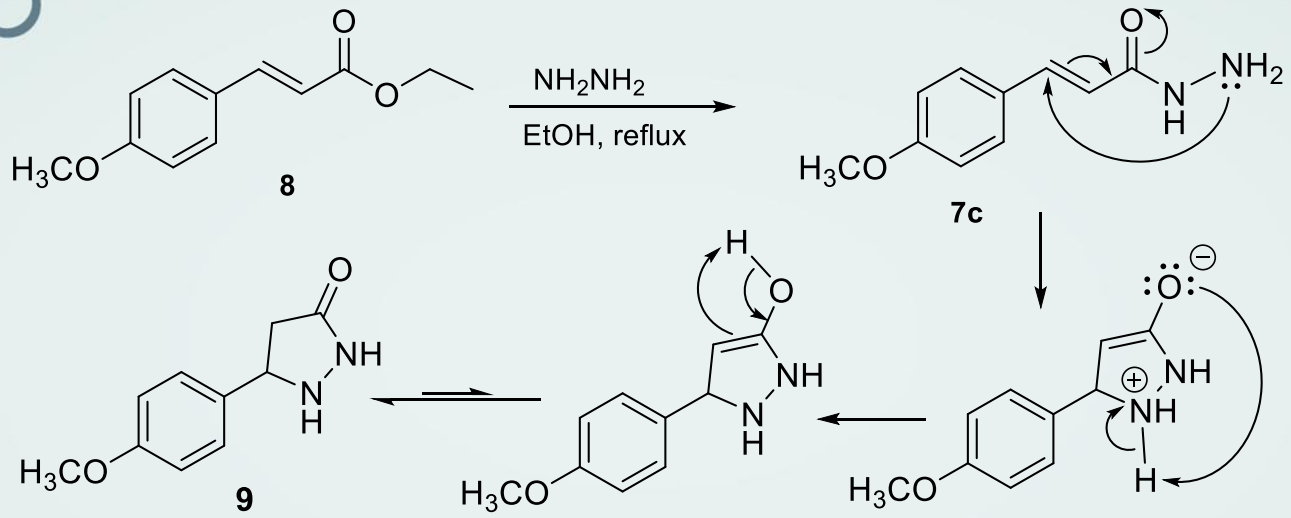
Synthesis of benzylidene cinnamoylhydrazides*



Scheme 1: Synthesis of benzylidene cinnamoylhydrazides 1–3. Reagents and conditions: (i) pyridine, piperidine, reflux; (ii) $SOCl_2$, CH_2Cl_2; (iii) NH_2NH_2, CH_3CN; (iv) R_2-benzaldehyde, AcOH, EtOH, reflux.

* M. Hossain et al., *Scientia Pharmaceutica* **2026**, 94, 86

Difficulty in the synthesis of cinnamoylhydrazide



Scheme 2: Cyclization of hydrazide 7c to give compound 9 under reflux conditions

* M. Hossain et al., *Scientia Pharmaceutica* **2026**, 94, 86

Structure determination of benzylidene cinnamoylhydrazides

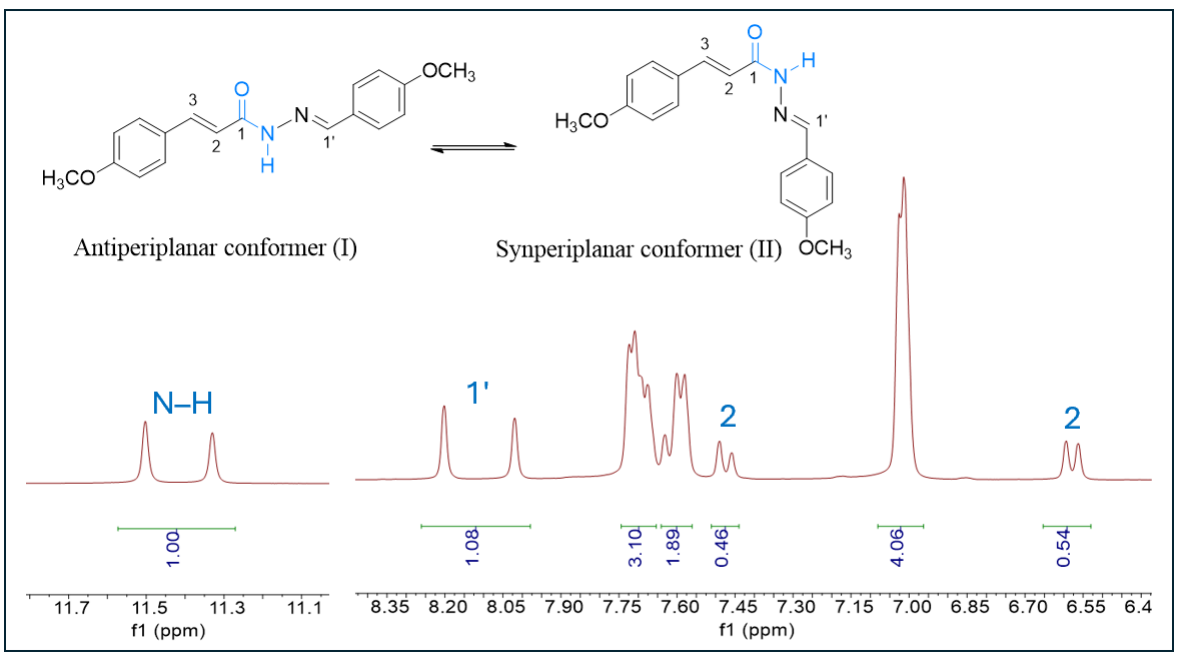


Figure 2: ^{1}H NMR spectrum of compound **1c**. Because of the presence of two conformers (I and II), the proton signals corresponding to N–H, 1′, and 2 appear as two signals of nearly equal intensity.

* M. Hossain et al., *Scientia Pharmaceutica* **2026**, 94, 86

Structure determination of benzylidene cinnamoylhydrazides

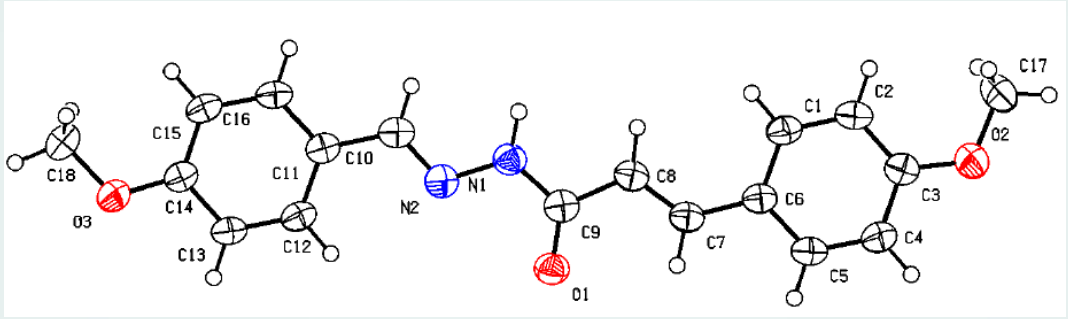


Figure 3: The X-ray crystallographic structure of compound **1c**.

* M. Hossain et al., *Scientia Pharmaceutica* **2026**, 94, 86

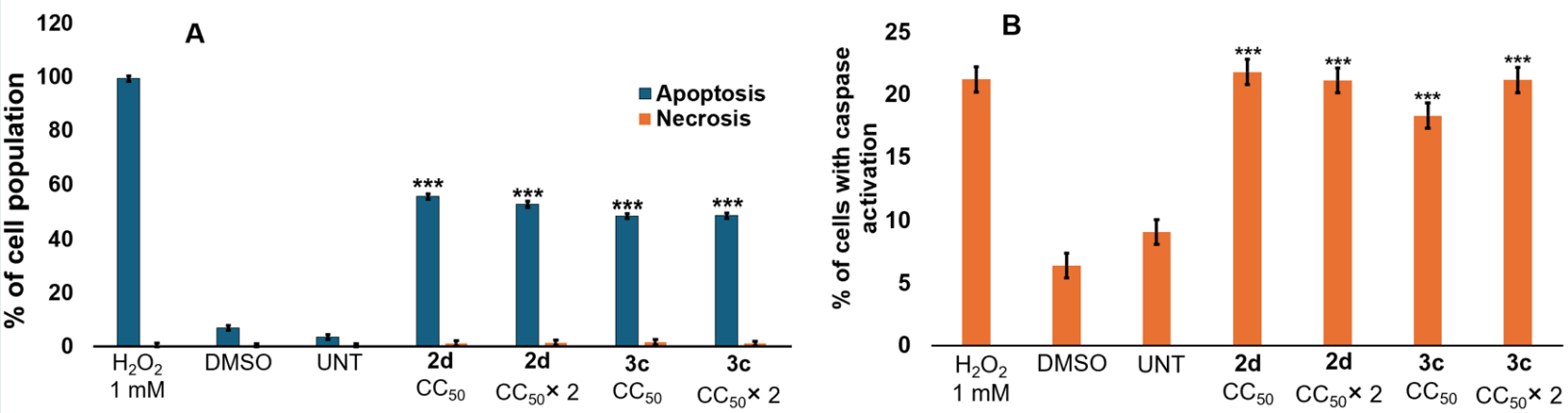
Cytotoxic evaluation of compounds 1a-d, 2a-d, 3a-c, and anticancer drugs against human T-cell lymphoma Jurkat and CEM cells after 48 hours.

Compounds	R_1	R_2	T-cell leukemia		Normal cell HS27 CC_{50} (μM)	Selectivity index (SI)
			Jurkat CC_{50} (μM)	CEM CC_{50} (μM)		
1a	H	H	30.05 ± 2.91	32.26 ± 1.67	-	
1b	4-Cl	4-Cl	> 40	> 40	-	
1c	4-OCH_3	4-OCH_3	> 40	> 40	-	
1d	4-CH_3	4-CH_3	> 40	> 40	-	
2a	H	4-Cl	> 40	> 40	-	
2b	H	4-F	> 40	> 40	-	
2c	H	4-OCH_3	37.49 ± 0.23	> 40	-	
2d	**H**	**4-CH_3**	**2.93 ± 0.35**	**1.59 ± 0.04**	**3.97 ± 0.18**	**1.76**
3a	4-Cl	H	> 40	24.45 ± 1.83	-	
3b	4-OCH_3	H	4.13 ± 0.11	15.35 ± 1.05	-	
3c	**4-CH_3**	**H**	**3.40 ± 0.36**	**3.09 ± 0.33**	**6.46 ± 0.15**	**1.99**
5-Fluorouracil	-	-	9.00 ± 0.15	35.00 ± 1.07	-	
Melphalan	-	-	> 40	> 40	-	

Evaluation of the antiproliferative effects of compounds **2d** and **3c** against representative cell lines from the NCI-60 human tumor cell line panel (HTS384 screen)

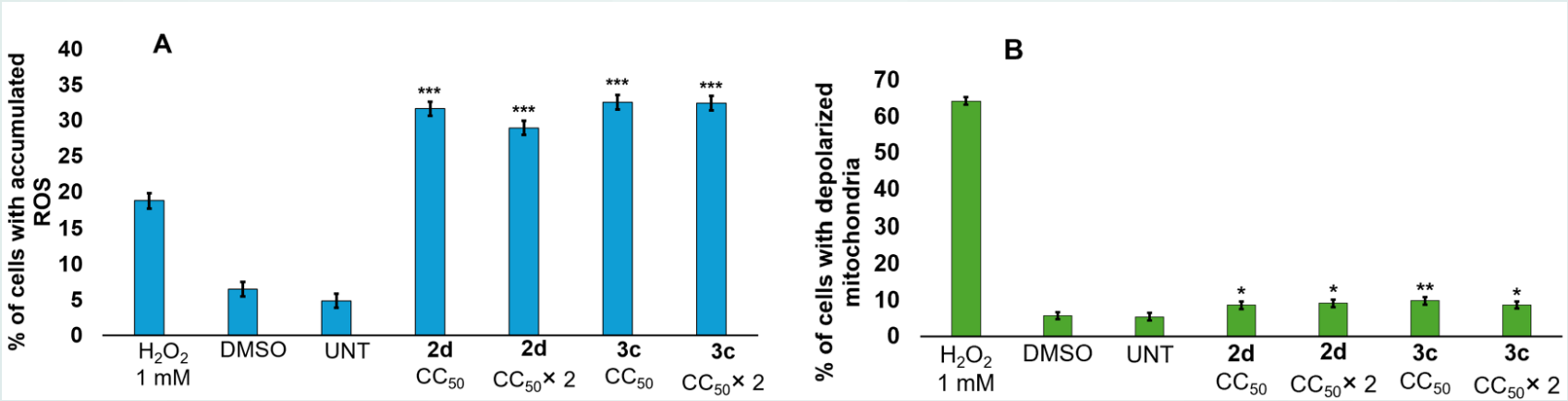
Panel	Cell Line	Compound 2d		Compound 3c	
		LC_{50} (μM)	GI_{50} (μM)	LC_{50} (μM)	GI_{50} (μM)
Leukemia	CCRF-CEM	4.26 ± 0.078	0.495 ± 0.094	5.08 ± 0.078	1.18 ± 0.021
	HL-60(TB)	0.731 ± 0.003	0.195 ± 0.009	3.55 ± 0.537	0.328 ± 0.004
	K-562	19.5 ± 6.44	0.298 ± 0.059	80.5 ± 21.4	0.327 ± 0.022
	MOLT-4	0.746 ± 0.142	0.206 ± 0.005	2.98 ± 1.20	0.312 ± 0.093
	RPMI-8226	0.877 ± 0.153	0.177 ± 0.004	1.24 ± 0.445	0.188 ± 0.039
	SR	0.593 ± 0.001	0.145 ± 0.003	0.610 ± 0.003	0.160 ± 0.002
Non-Small Cell Lung Cancer	NCI-H226	55.8 ± 49.9	0.685 ± 0.285	66.1 ± 45.8	1.98 ± 2.16
	NCI-H460	20.4 ± 12.4	0.328 ± 0.031	29.9 ± 18.5	0.370 ± 0.008
	NCI-H522	0.817 ± 0.066	0.171 ± 0.031	0.760 ± 0.050	0.155 ± 0.013
Colon Cancer	COLO 205	4.26 ± 0.827	0.326 ± 0.086	5.05 ± 1.18	0.444 ± 0.180
Melanoma	MDA-MB-435	1.40 ± 0.665	0.159 ± 0.020	5.22 ± 5.82	0.198 ± 0.045
	SK-MEL-2	5.45 ± 6.43	0.192 ± 0.054	7.57 ± 3.25	0.284 ± 0.004
Ovarian Cancer	NCI/ADR-RES	2.33 ± 0.940	0.223 ± 0.008	3.97 ± 2.58	0.259 ± 0.099
Breast Cancer	HS 578T	7.22 ± 1.31	0.500 ± 0.251	6.98 ± 2.31	0.682 ± 0.917

Compounds **2d** and **3c** induce apoptotic cell death and caspase activation in CEM cells.



* M. Hossain et al., *Scientia Pharmaceutica* **2026**, 94, 86

Compounds **2d** and **3c** increase ROS accumulation and mitochondrial membrane depolarization in CEM cells.



* M. Hossain et al., *Scientia Pharmaceutica* **2026**, 94, 86

Benzylidene hydrazides of phthalic acid as cytotoxic agents*

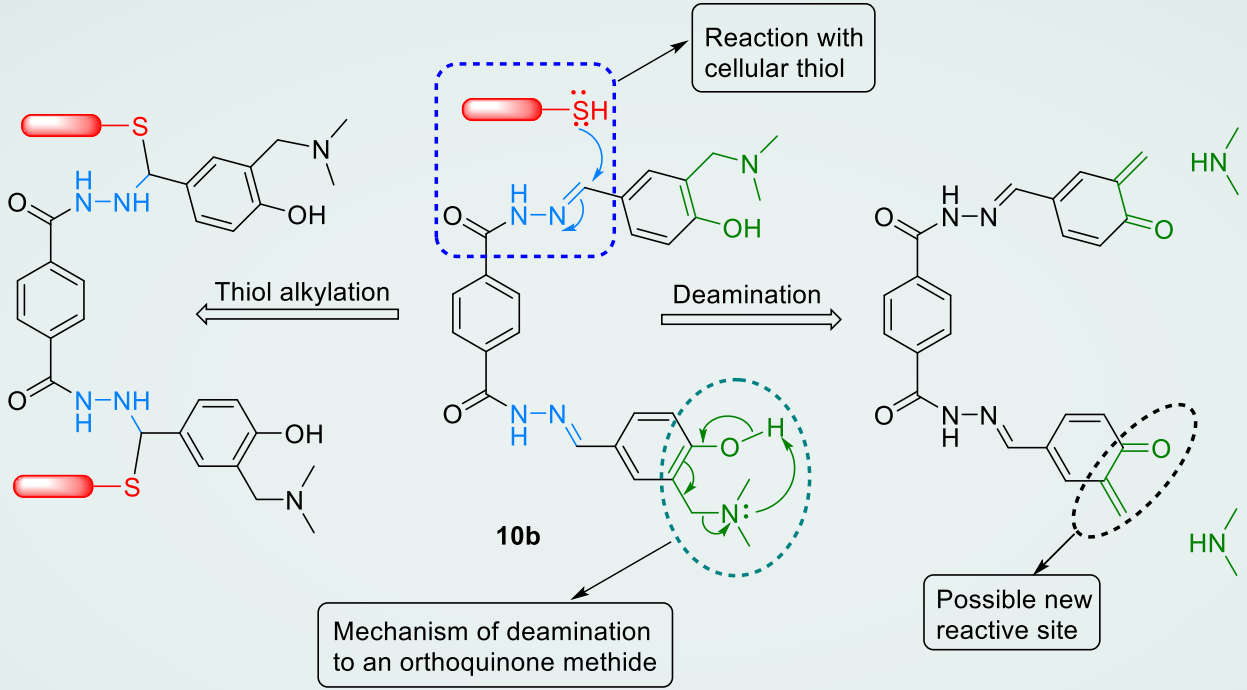


Figure: Possible modes of action of a representative compound **10b** with cellular constituents.

*M. Hossain et al., *Pharmazie* 77 (2022) 90-94

Benzylidene hydrazides of phthalic acid as cytotoxic agents*

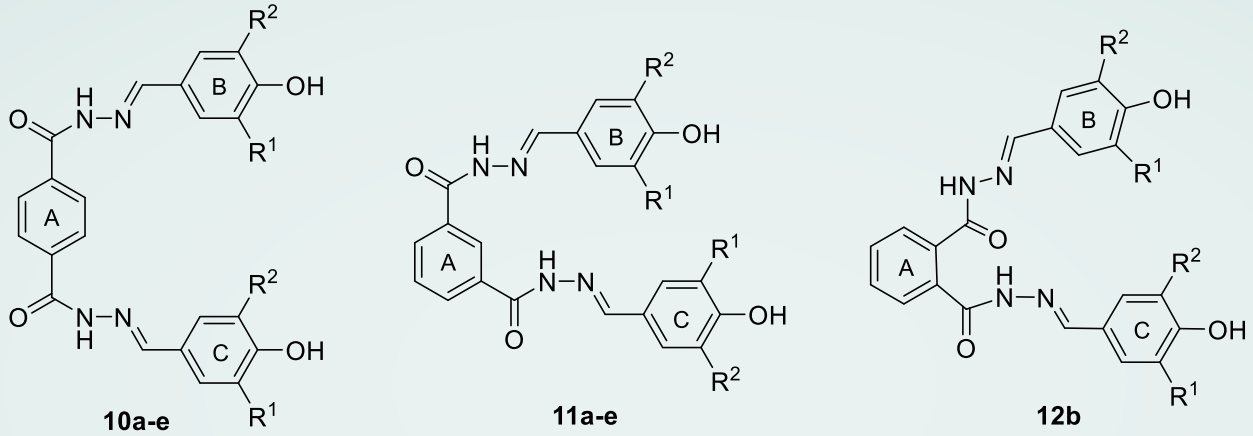


Figure: Structures of three series of hydrazides **10-12**. **a**: $R^1 = R^2 = H$; **b:** $R^1 = H$, $R^2 = CH_2N(CH_3)_2$; **c:** $R^1 = R^2 = CH_2N(CH_3)_2$; **d:** $R^1 = H$, $R^2 = CH_2N^+(CH_3)_3I^-$; **e:** $R^1 = R^2 = CH_2N^+(CH_3)_3I^-$.

Another objective was to investigate whether there is a correlation between cytotoxic potencies and the relative location of the two benzylidene hydrazide groups attached to ring A.

*M. Hossain et al., *Pharmazie* 77 (2022) 90-94

Acknowledgement to Students

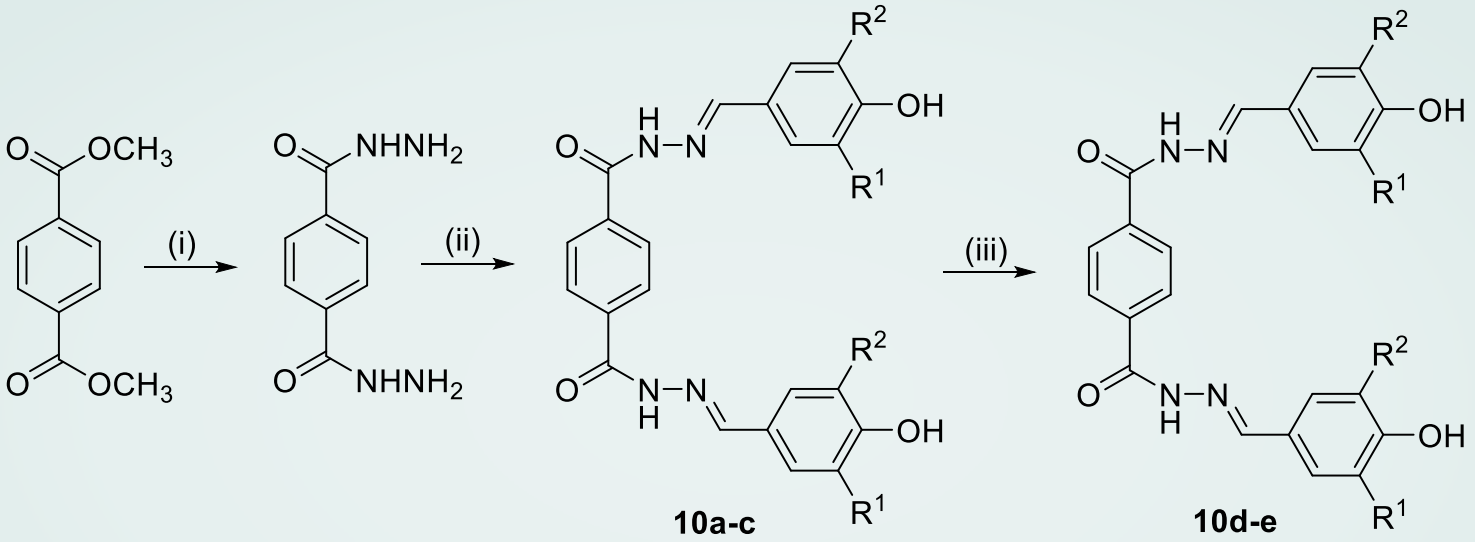
Skyler Hall

Payton Wiggington

Shayne Roth

Ross Filbrun

General synthesis of Benzylidene phthaloylhydazides *



Scheme 1: Synthesis of series **10**. The reagents are as follows: (i) NH_2NH_2. H_2O, Ethanol, reflux at 85 °C; (ii) Appropriate 4-hydroxybenzaldehyde, AcOH, Ethanol, reflux at 85 °C; (iii) CH_3I, DMSO.

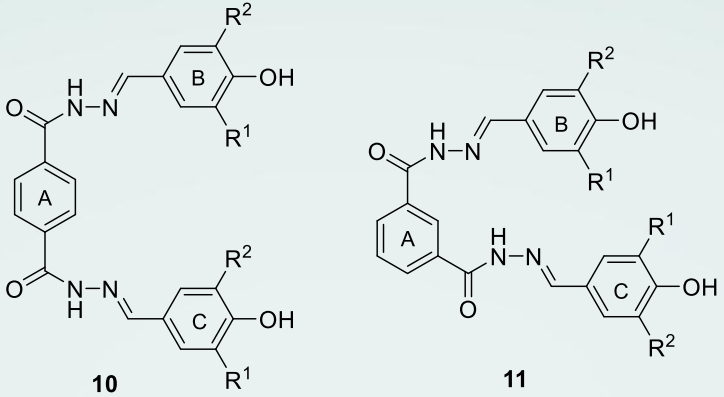
*M. Hossain et al., *Pharmazie* 77 (2022) 90-94

Cytotoxic evaluation of benzylidene hydrazides against various cells*

*M. Hossain et al., *Pharmazie* 77 (2022) 90-94

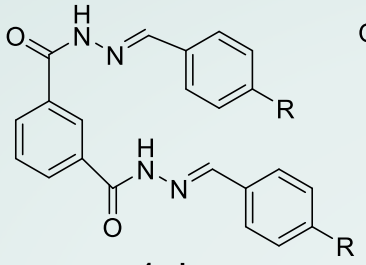
Compound	Colon Cancer cells				Non-malignant colon cells CRL1790 IC_{50} (μM)
	HCT116		HT29		
	IC_{50} (μM)	SI	IC_{50} (μM)	SI	
10a	0.17 ± 0.08	27.4	0.04 ± 0.01	116	4.65 ± 1.09
10b	4.09 ± 0.86	3.43	0.01 ± 0.00	1402	14.0 ± 2.96
10c	0.03 ± 0.01	94.3	0.42 ± 0.28	6.74	2.83 ± 0.70
10d	1.65 ± 0.20	3.09	0.20 ± 0.01	25.5	5.10 ± 0.24
11a	0.23 ± 0.09	288	14.1 ± 2.13	4.72	66.3 ± 2.43
11b	0.27 ± 0.17	98.4	0.36 ± 0.18	73.8	26.6 ± 1.90
11c	0.21 ± 0.13	120	0.15 ± 0.02	169	25.3 ± 1.89
11d	0.54 ± 0.21	29.6	0.19 ± 0.08	84.3	16.0 ± 0.16
5-Fluorouracil	4.22 ± 0.43	6.59	14.2 ± 0.60	1.95	27.8 ± 1.17

Significance of benzylidene hydrazides



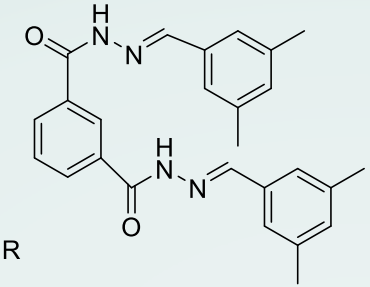
- The potency displayed by most of the synthesized hydrazides is highly impressive.
- These compounds are much less toxic to CRL1790 non-malignant cells giving rise to huge selectivity indices in many cases.
- The cytotoxic data against HCT116 cells suggested that greater potencies were found with the analogs in series **11**. That is for isophthalohydrazides (1,3-substituted)
- However, for HT29 cells, a firm comparison could not be made for series **10** and **11** as mixed results were obtained.

Benzylidene isophthaloylhydazides as cytotoxic agents*

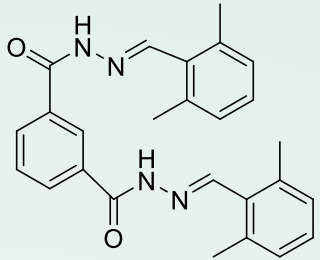


1a-h

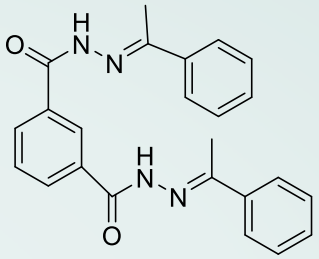
a: R=OH, **b:** R=Cl, **c:** R=F, **d:** R=CH_3, **e:** R=OCH_3, **f:** R=H, **g:** R=$N(CH_3)_2$, **h:** R=NO_2



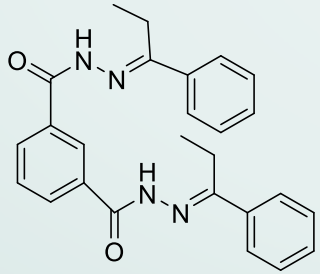
1i



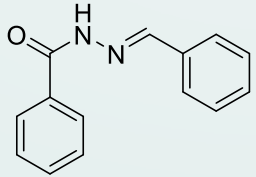
1j



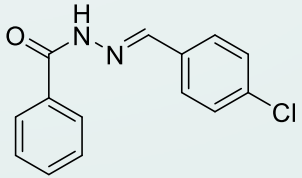
1k



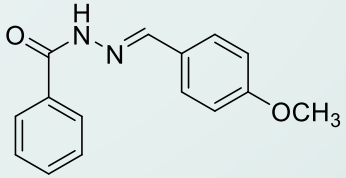
1l



1m



1n



1o

*M. Hossain et al., *Anticancer Research* 46: 1183-1195 (2026)

Tumor-specificity of compounds **1a-o** and anticancer drugs.

Compounds	Human OSCC cell lines			Normal oral cells			SI^b
	Ca9-22 CC_{50} (μM)	HSC-2 CC_{50} (μM)	Mean (A) CC_{50} (μM)	HGF CC_{50} (μM)	HPC CC_{50} (μM)	Mean (B) CC_{50} (μM)	(B/A)
1a (R = OH)	53.93 ± 5.90	60.12 ± 8.16	57.03	200.00	200.00	200.00	3.5
1b (R = Cl)	10.94 ± 1.18	4.93 ± 1.58	7.94	13.70 ± 5.32	0.57 ± 0.03	7.13	0.9
1c (R = F)	5.62 ± 1.46	0.40 ± 0.20	3.01	9.19 ± 4.35	0.53 ± 0.05	4.86	1.6
1d (R = CH_3)	38.24 ± 2.94	3.64 ± 1.17	20.94	200.00	4.05 ± 0.31	102.03	4.9
1e (R = OCH_3)	22.49 ± 9.28	2.11 ± 1.51	12.30	200.00	3.18 ± 2.42	101.59	8.3
1f (R = H)	5.22 ± 2.39	3.50 ± 1.16	4.36	200.00	10.16 ± 4.14	105.08	24.1
1g (R = $N(CH_3)_2$)	1.48 ± 0.13	1.03 ± 0.53	1.25	2.01 ± 0.45	1.47 ± 0.36	1.74	1.4
1h (R = NO_2)	1.67 ± 0.19	5.05 ± 2.26	3.36	200.00	200.00	200.00	59.5
1i	200.00	2.98 ± 1.25	101.49	12.48 ± 6.27	9.97 ± 3.83	11.22	0.1
1j	0.57 ± 0.01	0.21 ± 0.02	0.39	0.44 ± 0.15	0.38 ± 0.07	0.41	1.0
1k	1.53 ± 0.14	0.54 ± 0.03	1.04	2.03 ± 0.27	3.42 ± 0.11	2.73	2.6
1l	13.42 ± 3.97	1.00 ± 0.67	7.21	4.39 ± 2.62	4.72 ± 1.24	4.55	0.6
1m	200.00	200.00	200.00	200.00	200.00	200.00	1.0
1o	110.87±6.51	187.99±11.6	149.43	200.00	200.00	200.00	1.3
Melphalan	22.28 ± 1.68	8.43 ± 0.56	15.36	133.00	118.05	125.53	8.2
Cisplatin	8.11 ± 0.24	6.40 ± 0.34	7.25	133.00	68.81 ± 1.09	100.90	13.9

Neurotoxicity benzylidene isophthaloylhydazides and anticancer drugs*.

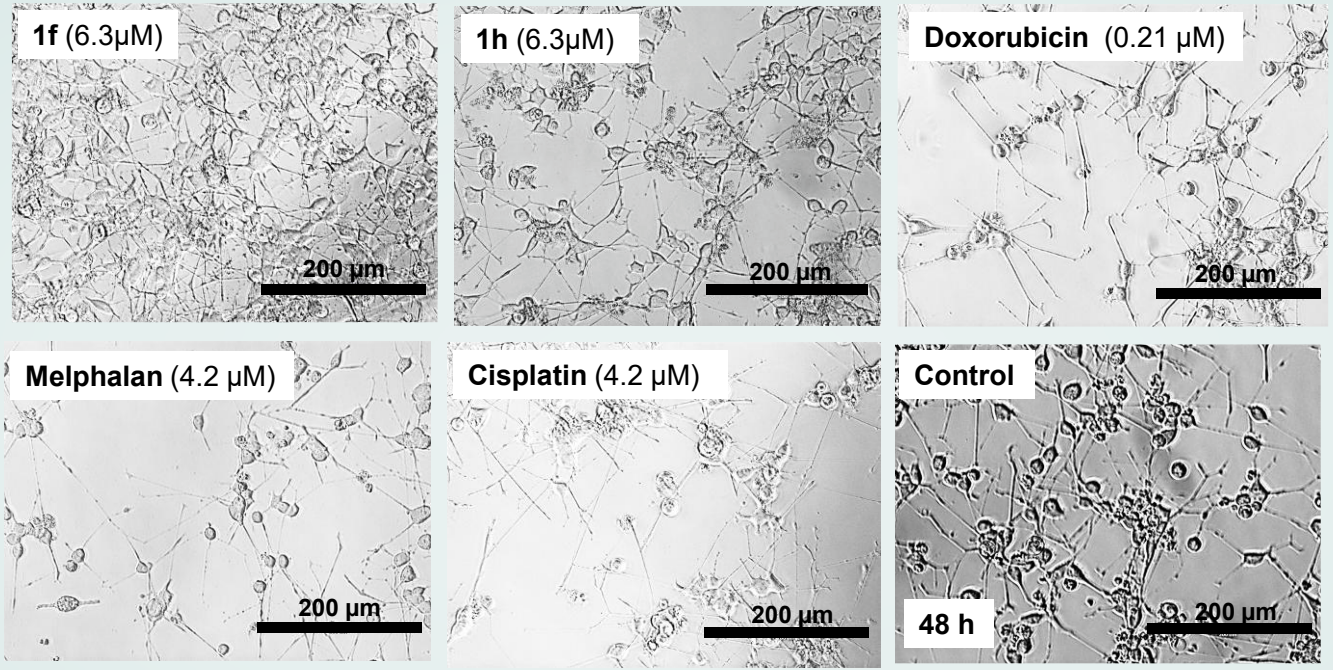
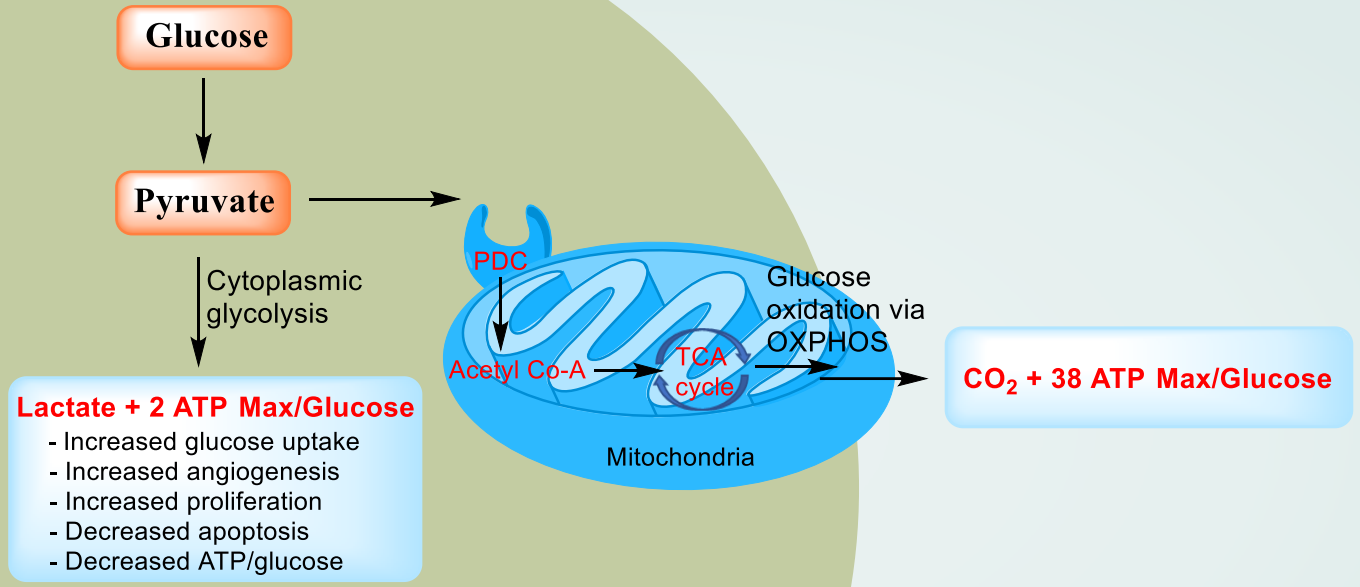


FIGURE 6 Doxorubicin, melphalan and cisplatin, but not **1f** and **1h**, induced neurites disruption.

*M. Hossain et al., *Anticancer Research* 46: 1183-1195 (2026)

Oxidative Phosphorylation Vs Cytoplasmic Glycolysis

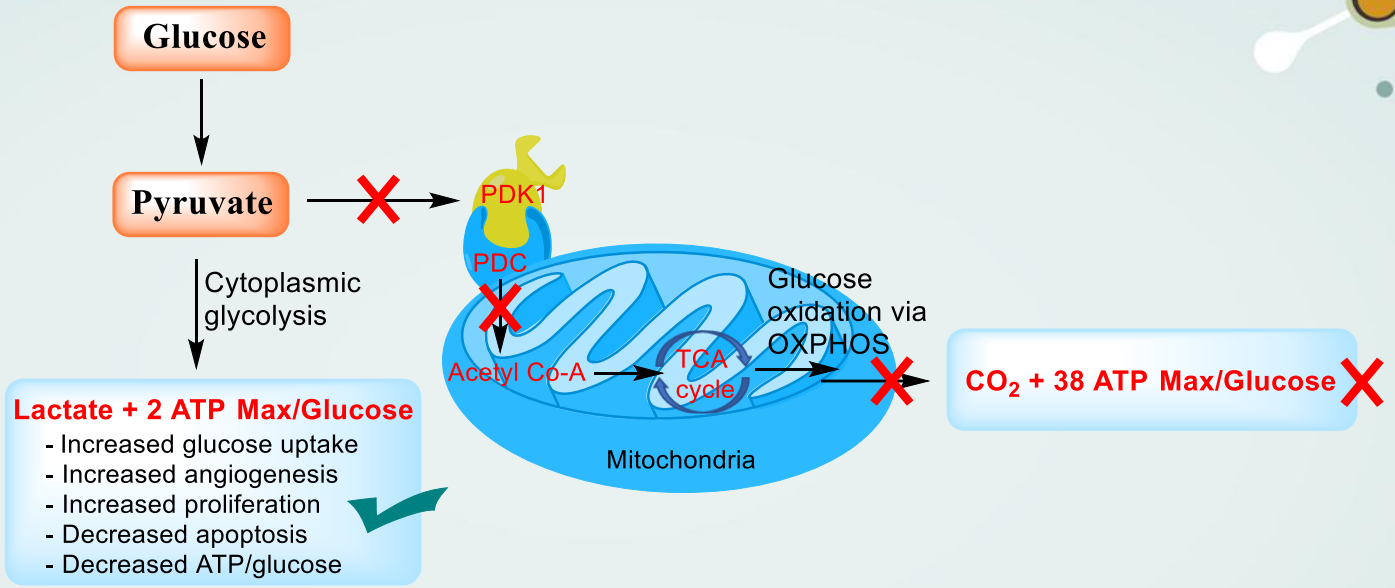


Metabolizing glucose via cytoplasmic glycolysis involves a thermodynamic trade-off between the yield and rate of ATP production:

- ATP can be rapidly synthesized by glycolysis, up to 100 times faster than OXPHOS
- but the energetic yield of glycolysis is very low, as it produces 18 times less ATP than OXPHOS

*M. Hossain et al., *Archiv Der Pharmazie,* **2022**, 355 (11), e2200236

Back to the Cancer Cell's Metabolism



Otto Warburg in the 1920s stated that for ATP production cancer cells tend to transform glucose into lactate via glycolysis even under aerobic conditions.

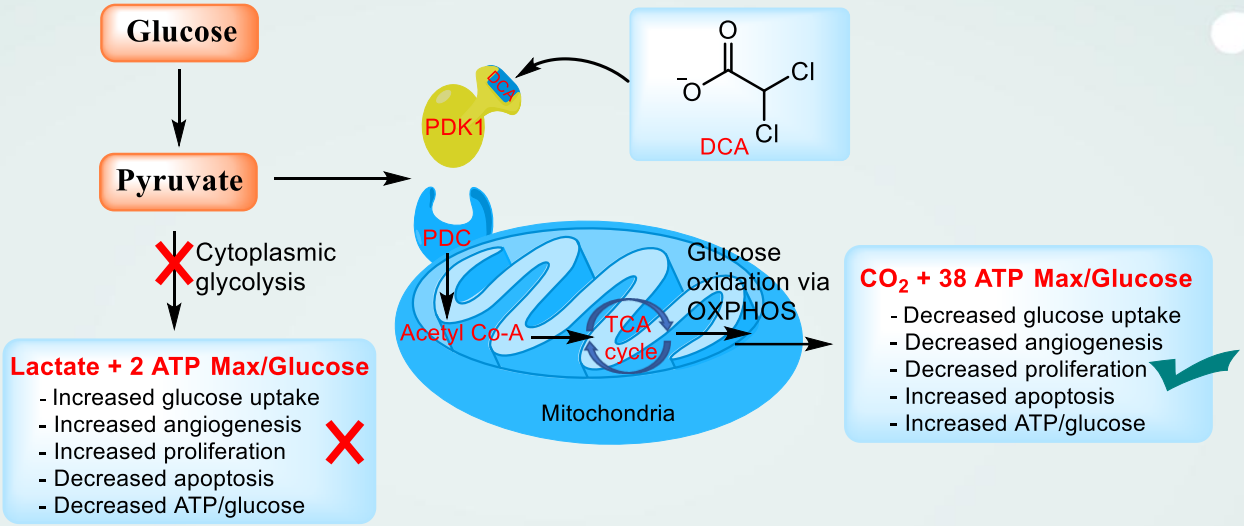
*M. Hossain et al., *Archiv Der Pharmazie,* **2022**, 355 (11), e2200236

Metabolic pathway preferred by cancer cells

- For oxidative phosphorylation inside mitochondria, pyruvate must first be converted to acetyl-CoA in a series of reactions facilitated by the pyruvate dehydrogenase complex (PDC).
- PDC inhibition can occur by pyruvate dehydrogenase kinase-1 (PDK1).
- PDK1 is commonly overexpressed in cancer cells.
- PDC inhibition by PDK1's stops acetyl-CoA formation from pyruvate, which prevents oxidation from occurring in the TCA cycle and inhibits oxidative phosphorylation.

*M. Hossain et al., *Archiv Der Pharmazie,* **2022**, 355 (11), e2200236

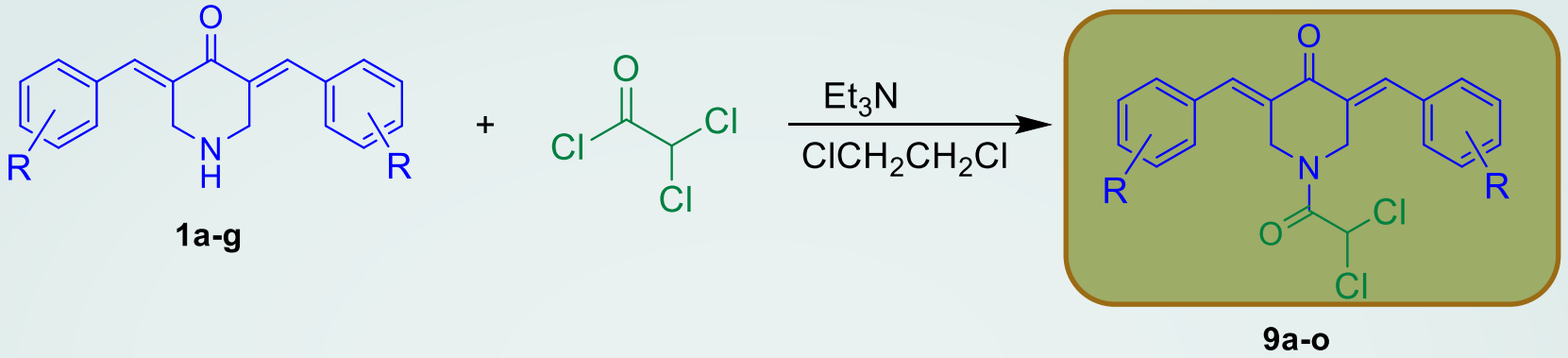
Alteration of Cancer Metabolism by DCA



- Dichloroacetate (DCA) can inhibit PDK1 which activates PDC and subsequently facilitates OXPHOS.
- DCA can reverse the glycolytic pathway by activating a metabolic switch from aerobic glycolysis to oxidative phosphorylation

*M. Hossain et al., *Archiv Der Pharmazie,* **2022**, 355 (11), e2200236

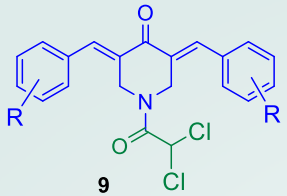
Synthesis of Compounds 9a-o as novel cytotoxins*



- The objective was to attach the dichloroacetyl group to the cytotoxic warhead **1**.
- The dichloroacetate amides **9** will hydrolyze *in vitro* and *in vivo* releasing compounds **1** and dichloroacetate as the active drug components.
- Compounds **1** will act as thiol alkylators because of the 1,5-diaryl-3-oxo-1,4-pentadienyl group and preferentially sensitize tumors in contrast to normal cells.
- DCA will inhibit the enzyme PDK resulting the alteration of tumours metabolism adversely.

*M. Hossain et al., *Bioorganic & Medicinal Chemistry Letters* 30 (2020) 126878

Evaluations of series **9** against HCT116 colon cancer Cell lines*

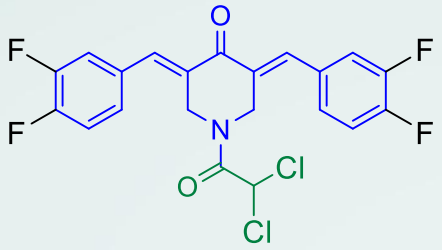


9

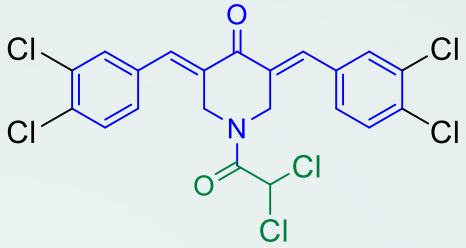
*M. Hossain et al., *Bioorg & Med Chem Lett* 30 (2020) 126878

Compound	Substituent R	Cytotoxicity; IC_{50} (μM)		Selectivity indices: $\frac{(IC_{50})_{CRL1790}}{(IC_{50})_{HCT116}}$
		Human HCT116 colon cancer cells	Non-malignant CRL 1790 colon cells	
9a	H	0.23 ± 0.053	12.45 ± 0.50	54
9b	4-CH_3	0.03 ± 0.005	16.22 ± 0.61	541
9c	4-F	0.05 ± 0.020	14.59 ± 0.67	292
9d	4-NO_2	0.03 ± 0.006	11.05 ± 0.76	368
9e	3,4-F_2	0.06 ± 0.023	11.07 ± 0.64	184
9f	3,4-Cl_2	0.04 ± 0.005	12.21 ± 0.56	305
9g	3,4,5-$(OCH_3)_3$	0.04 ± 0.010	15.06 ± 0.56	376
5-Fluorouracil	---	4.016 ± 0.88	18.69 ± 0.82	4.7

More significance of dichloroacetyl amides 9*



9e



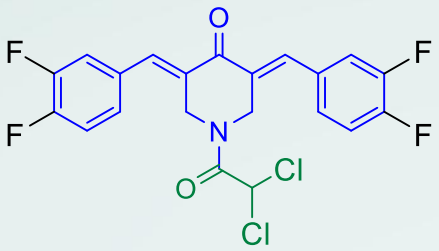
9f

- Compounds **9e** and **9f** were further investigated against various human cancer cell lines such as breast, pancreatic, leukemia/lymphoma, colon, and fibroblasts.
- In all cases, both compounds showed remarkable cytotoxic activity with CC_{50} in the low micromolar to nanomolar concentration range.

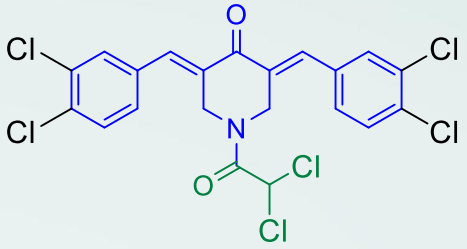
*M. Hossain et al., *Medicines,* **2022**, 9(6), 35

* R. M. Swain et al., *Investigational New Drugs*, **2022**, 40, 905–921

More significance of dichloroacetyl amides 9*



9e



9f

- Both **9e** and **9f** induced accumulation of reactive oxygen species (ROS), mitochondrial depolarization, and activated caspase-3/7 in lymphoma and colon cell lines.
- Both compounds induced cell death via the intrinsic apoptotic pathway.
- Both compounds caused cell cycle alteration in lymphoma and colon cell lines.

*M. Hossain et al., *Medicines,* 2022, 9(6), 35
* R. M. Swain et al., *Investigational New Drugs*, **2022**, 40, 905–921

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Do you have any questions?

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