Natural Resources for Human Health

visaga publishing house

Original Research

View Article Online

Check for updates

Received 14 July 2021
Revised 30 August 2021
Accepted 01 September 2021
Available online 28 November 2021

Edited by Ricardo DE Albuquerque

KEYWORDS:
Flavonoids
Mannich Base
Phenolic compounds
HeLa
MCF7

Natr Resour Human Health 2022; 2 (2): 100-106
https://doi.org/10.53365/nrfhh/141866
eISSN: 2583-1194

Synthesis and antitumor activities of novel Mannich base derivatives derived from natural flavonoids

Huma Aslam Bhatti [1,*], Rubina Rubina [1], Faisal Rashid [2], Sumera Zaib [2], Jamshed Iqbal [2], Abdul Hameed [1]

[1]H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi-75270, Karachi Pakistan

[2]Centre for Advanced Drug Research, COMSATS University Islamabad, Abbottabad Campus, Abbottabad-22060, Pakistan

ABSTRACT: In our current study, a series of reactions with isolated natural flavonoids (2-phenylchromen-4-one) and flavanone (2,3-dihydro-2-phenylchromen-4-one) through Mannich base was carried out by a one-pot three-component reaction. Their structure-activity relationship study (SAR) reveals the anticancer activity of natural compounds and their Mannich bases. The flavones were substituted by imine at position C-8, while in the flavanones, the reaction takes place at positions C-8 and C-3. Spectroscopic techniques characterized all the isolated and newly synthesized derivatives. Anticancer activity was checked on HeLa and MCF-7 (cancer cell lines) and BHK-21 (normal cell line). Using propidium iodide (PI) and DAPI staining as fluorescence microscopic imaging was confirmed the Apoptotic effect of potent compound. Further, it was evaluated by cell cycle analysis through flow-cytometry, reactive oxygen species and lactate dehydrogenase production. The caspase-9 and -3 activity were estimated by mitochondrial membrane potential. Derivative of naringenin, ((2S)-4′,5,7-Trihydroxyflavan-4-one) where reactions occur at position C-3 were active than others.

1. INTRODUCTION:

Secondary metabolites represent phenolic compounds as are main class. Among these compounds, flavonoids are the most common and widely distributed class of plant phenolics (Brodowska, 2017); about 10,000 flavonoids have been reported (Santos et al., 2017). Flavonoids are classified into ten chemical groups, and different flavonoids (over 5000) have been discovered to date (Khan et al., 2014).

According to the molecular structure of flavonoids, they are classified into six different classes: flavonols, flavones, flavanols, flavonones, isoflavonones and anthocyanins (Ross & Kasum, 2002). Flavonoids availability in plants plays many physiological roles, as they exhibit anti-oxidant, anti-cancer, anti-bacterial, and anti-ageing properties (Liu et al., 2012). Many isolated flavonoids exhibit different pharmacological activities including anti-cancer, (Zhang et al., 2017) anti-inflammatory, (Saini et al., 2017), anti-allergic, (Tanaka et al., 2003), anti-viral, (Sanchez et al., 2000; Sánchez-Roque et al., 2017) and anti-oxidant activities (Braca et al., 2002). Some common uses of pure flavonoids are to treat cardiovascular and gastrointestinal disorders. Flavones and flavanones found in different fruits and vegetables exhibited potent biological activity, including anti-cancer, anti-diabetic, anti-inflammatory, anti-hypertension, relief from obesity, and health effects on cardiovascular disease (Pandey et al., 2015).

In this study, we use the concept of natural product-based drug discovery since nitrogen-containing heterocyclic compounds play a vital role in the medicinal field (Liaqat et al., 2017). The nitrogen moiety was introduced in the plant-based compounds to enhance its biological activity or search for other medicinally essential compounds. For this purpose, we used the mannich reaction method to form a carbon-nitrogen (C-N) bond. Since mannich reaction obtains importance in the chemical and pharmaceutical field (Armstrong et al., 1996). Mannich reaction is the three-component reaction of amine (primary or secondary), aldehyde and carbonyl compounds that form mannich bases (Haji, 2016), which shown different biological activities such as anti-cancer (Lopes et al., 2004), anti-inflammatory (Sujith et al., 2009), anti-bacterial (Kantlam et al., 2015), anti-fungal (Ashok et al., 2007), anti-tubercular (Joshi et al., 2004), anti-malarial (Lopes et al., 2004) and anti-analgesic drug (Malinka et al., 2005).

Neoplasm, a group of more than 270 diseases affecting all age groups and almost all body tissues, organs, and systems, is a multi-factorial, multi-step, multistage accruing illness that initiates after multiple genetic and epigenetic modifications in the normal nuclear makeup of cells usually characterized by

* Corresponding author.
E-mail address: habchemist31@gmail.com (Huma Aslam Bhatti)

unchecked, unusual and uncontrolled proliferation (Hanahan & Weinberg, 2011; Hassanpour & Dehghani, 2017; Heng et al., 2010). Tumorigenesis is a multifaceted and multistage process that involves the unusual multiplication of a group of cells in a specific body part after a significant driver mutation. The cellular DNA repair mechanism fails to fix such transformations. That sort of cells increases their population and pass on mutated genetic material to lineage (Kim, 2015). Further mutations in these cells lead to heterogeneity, a feature of neoplasm. Such cells produce larger populations, start affecting nearby cells, enlarge their size, develop new blood vessels and consequently invade other body parts or systems, a characteristic of malignancy known as metastasis. Metastatic malignancy is life-threatening and requires rigorous treatment, intensive care and critical monitoring of untoward effects (Kim, 2015).

Surgical removal, radiation therapy, immunotherapy, hormonal starvation therapy, targeted therapy, stem cell grafting, and chemotherapy are the treatment options for cancer (Miller et al., 2016). Chemotherapy is the most widely adopted treatment strategy for cancer, either alone or with other therapies. Both natural and synthetic compounds are currently in practice to combat cancer, but conventional chemotherapeutic approaches cannot control the incidence of most cancer types. Introducing novel compounds with improved pharmacological actions is a need of the hour. Synthetic chemistry has an edge to synthesize natural compounds on a larger scale economically, thus helping in the continuous supply of life-saving medicines (Dua et al., 2011; Maccoss & Baillie, 2004). Compounds of natural origin have always been a mainstay of lead compounds (Gordaliza, 2007). Organic chemical synthesis provides an opportunity to manufacture and modify natural leads by structural changes and substitutions to analyze such compounds extensively in search of more specific, effective and safer chemotherapeutics (Blagosklonny, 2005; Shang & Tan, 2005; Yeh & Lim, 2007). In the current study, derivatives of flavons and flavanones were synthesized by a Mannich reaction. In flavones, the reaction occurs at the C-8 position. This is because of the π–conjugation of the hydroxyl group at C-5 and C-7 in ring A (Liu et al., 2012). While in flavanone, the reaction happens at C-8 and C-4 positions because of the enolizable proton at that position. The MTT cell viability assay was used to test the anticancer potential of these flavones, flavanones, and derivatives on HeLa and MCF-7 cancer cells. The apoptotic impact was validated using propidium iodide (PI) and DAPI staining. It was also tested for cell cycle, reactive oxygen species, lactate dehydrogenase, mitochondrial membrane potential, and caspase-9 and -3 activity.

2. MATERIALS AND METHODS

2.1. Experimental material

Naringenin and Chrysin were isolated from Callistephus chinensis and Flavanone from Ocimum basilicum. All other chemicals and solvents that were used in this research were purchased from Sigma Aldrich. NMR was recorded on Brucker. ^{1}HNMR and ^{13}CNMR spectrometers were operated at 400 and 150 MHz. The value of chemical shift and coupling constants were expressed in ppm and Hz. Mass spectra were recorded using EI and ESI-MS spectrometer.

Experimental material and procedure for anticancer activity was followed by previous procedure (Abbas et al., 2021; Lin et al., 2013; Rastogi et al., 2010; Scifo et al., 2004; Uddin et al., 2020)

2.2. Synthesis of Mannich base derivatives

2.2.1 Synthesis of Compound 2

In a two necked 250ml round bottom flask, Chrysin that is mentioned as compound 1 (100 mg, 0.39mmole) were dissolved in DMF (1ml), and Methanol (15ml) was added as a solvent, all the reagent kept on oil bath with constant stirring then dicyclohexylamine (600μl, 0.58mmole), formaldehyde solution (200μl, 1mmole) were added dropwise to the reaction mixture. The reaction was allowed to get the homogenized mixture. After 2 hours, yellow precipitates were formed, and precipitates were filtered through filter paper. The pure yellow color residue was collected with a 59.7% yield.

2.2.2 Synthesis of Compound 4

Flavanone (100mg, 0.45mmole) was dissolved in anhydrous DMF (1ml) and Methanol (15ml) in a two necked round bottom flask and immersed in an oil bath with constant stirring for 12 hours and then the solution of Dicyclohexylamine (700μl, 1.3mmole) and formaldehyde solution (50μl, 1.7 mmole) were added in a reaction mixture and kept in an oil bath to get homogenized mixtures and the overall reaction was monitored through TLC. After 12 hours, the reaction was halted and filtered through filter paper, followed by the collection and drying of the supernatant under decreased pressure. Purification of the product using PTLC (2 percent Methanol: 98 percent DCM) yielded compound 4 in 44 percent yield.

2.2.3 Synthesis of Compound 6 and 7

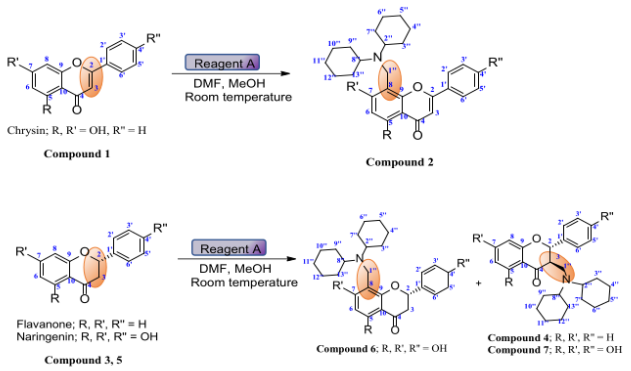
Naringenin (100mg, 0.37mmole), DMF (1ml) and Methanol (15ml) were taken in two necked round bottom flasks. The reaction was kept on an oil bath with continuous stirring for 2 hours, then Dicyclohexylamine (110μl, 0.5mmole) and formaldehyde solution (50μl, 1mmole) was added to the reaction mixture. After the completion of the reaction, the reaction mixture was filtered through filter paper, and the supernatant was collected. It was purified using column chromatography after solvents were evaporated at decreased pressure (ethyl-acetate and hexane), and we got the two compounds (6 and 7) with a 23% yield.

3. RESULT AND DISCUSSION

3.1. Synthesis of Mannich Bases

Flavones and flavanones are considered antioxidant compounds, and the Mannich reaction synthesized their derivatives.

For the structure-activity relationship, we checked all the isolated and their derivatives for anticancer activity. The compounds (mentioned 1, 3, and 5) were isolated from a plant source and used to synthesise compounds (mentioned 2, 4, 6 and 7) using dicyclohexylamine, formaldehyde, dimethylformamide, and methanol as solvent **Scheme 1** and **2** represents their synthesis.



Scheme 1. Scheme representing synthesis of mannich bases from flavones and flavanones

Reactant	Reagent	Time duration nd temperature	Product	% Yield
Compound 1	And	2 hr, Room temperature	Compound 2	59.7%
Compound 3	And	12 hr, Room temperature	Compound 4	44.7%
Compound 5	And	2 hr, Room temperature	Product 1 Compound 6	23%
			Product 2 Compound 7	23%

Scheme 2. Synthesis of mannich bases from flavones andflavanones

All the isolated and synthesized compounds were characterized by using UV, EI-MS, ^{1}HNMR, ^{13}CNMR techniques. The purity of compounds was determined by TLC. All synthesized mannich bases were purified by normal phase column chromatography except compound **2** purified by the precipitation method. The ^{1}HNMR data for the isolated compounds are presented in Table 1. The characteristic peak of the newly synthesized compounds is represented in Table 2 and in Appendix A.

Table 1
^{1}HNMR values of natural product in DMSO-d6 at 500 MHz.

Carbon no.	Compound 1 (^{1} HNMR)	Compound 3 (^{1} HNMR)	Compound 5 (^{1} HNMR)
1	-	-	-
2	-	4.06 (dd, J = 2.8, 13.1 Hz)	5.42 (dd, J = 2.8, 13.0 Hz)
3	6.95 (s)	3.06-3.13 (m, 2H)	2.66 (dd, J = 2.6, 16.8 Hz, H-3a), 3.09 (dd, J = 12.8, 17.2 Hz, H-3b)
4	-	-	-
5	12.81 (s, Ar-OH)	7.91 (dd, J = 1.6, 8.0 Hz)	12.13 (s, Ar-OH)
6	6.21 (d, J = 2.0 Hz)	7.14-7.15 (m)	5.87 (s)
7	10.89 (s, Ar-OH)	7.14-7.15 (m)	10.76 (s, Ar-OH)
8	6.51 (d, J = 2.0 Hz)	7.07 (dd, J = 1.6, 8.0 Hz)	5.87 (s)
9	-	-	-
10	-	-	-
1'	-	-	-
2'	8.05 (d, J = 8.0 Hz)	7.56-7.59 (m)	7.30 (d, J = 8.4 Hz)
3'	7.57-7.59 (m)	7.43-7.46 (m)	6.78 (d, J = 8.4 Hz)
4'	7.57-7.59 (m)	7.43-7.46 (m)	9.56 (s, Ar-OH)
5'	7.57-7.59 (m)	7.43-7.46 (m)	6.78 (d, J = 8.4 Hz)
6'	8.05 (d, J = 8.0 Hz)	7.56-7.59 (m)	7.30 (d, J = 8.4 Hz)

3.2. Anticancer activity

All the isolated and derivatives cell viability was explored on HeLa and MCF-7 (cancer cell lines) and BHK-21 (normal cell line). Compounds 1,6 and 7 showed more than 50% inhibition against HeLa, MCF-7 and BHK-21 cells, as shown in Table 3. Compound 7 was selected to investigate its mechanism of action through various assays, as shown in Figure 1 and 2.

Fluorescence DCF oxidation in HeLa cells induced by 2 × IC_{50} values of compound 7, cisplatin, and untreated cells following exposure to 2',7'-dichlorofluorescein diacetate. The apoptotic impact of compound 7 was demonstrated in Figure 3.

Caspase 8, 9 initiates the executioner caspase 3, 6 and 7 that has the prompt part in activating apoptosis. Compound 7 demonstrated a reduction in the mitochondrial membrane potential in HeLa cells, indicating that it may have triggered apoptosis via the intrinsic mechanism. (Figure 4, 5 and 6).

Using propidium iodide PI) and DAPI staining as fluorescence microscopic imaging was confirmed the apoptotic effect

Table 2
^{1}H and ^{13}CNMR values of mannich bases in DMSO-d6 at 500 and 150 MHz.

C no.	Compound 2 (^{1}HNMR)	Compound 2 (^{13}CNMR)	Compound 4 (^{1}HNMR)	Compound 6 (^{1}HNMR)	Compound 7 (^{1}HNMR)
1	-	-	-	-	-
2	-	162.8	5.55 (d, J = 6.4Hz)	5.42 (dd, J = 2.8, 13.0 Hz)	5.29 (d, J = 3.6Hz)
3	6.64 (s)	104.2	4.06-4.08 (m)	2.66 (dd, J = 2.6, 16.8 Hz, H-3a), 3.09 (dd, J = 12.8, 17.2 Hz, H-3b)	2.66-3.19 (m)
4	-	181.6	-	-	-
5	12.81 (s, Ar-OH)	157.9	7.91 (dd, J = 1.6, 8.0 Hz)	12.5 (s, Ar-OH)	12.3 (s, Ar-OH)
6	6.21 (s)	94.9	7.14-7.15 (m)	5.86 (s)	5.87 (s)
7	10.89 (s, Ar-OH)	170	7.14-7.15 (m)	9.5 (s, Ar-OH)	9.48 (s, Ar-OH)
8	-	102.2	7.07 (dd, J = 1.6, 8.0 Hz)	-	5.87 (s)
9	-	156.9	-	-	-
10	-	105.1	-	-	-
1'	-	132	-	-	-
2'	7.97 (d, J = 7.6Hz)	126.5	7.56-7.59 (m)	7.27 (d, J = 8.4 Hz)	7.27 (d, J = 8.4Hz)
3'	7.57-7.59 (m)	129.4	7.43-7.46 (m)	6.76 (d, J = 8.4 Hz)	6.67 (d, J = 8.4Hz)
4'	7.57-7.59 (m)	126.5	7.43-7.46 (m)	-	9.42 (s, Ar-OH)
5'	7.57-7.59 (m)	129.4	7.43-7.46 (m)	6.76 (d, J = 8.4 Hz)	6.67 (d, J = 8.4Hz)
6'	7.97 (d, J = 7.6Hz)	126.5	7.56-7.59 (m)	7.27 (d, J = 8.4 Hz)	7.27 (d, J = 8.4Hz)
1"	4.35 (s)	42.7	2.33-2.35 (m)	4.65 (s)	2.64-2.65 (m)
2"	2.04-2.5 (m)	58.5	3.12-3.15 (m)	2.58-2.60 (m)	3.03-3.05 (m)
3"	1.71-1.75 (m)	29.2	1.85-1.89 (m)	1.77-1.81(m)	1.79-1.83 (m)
4"	1.26-1.32 (m)	25.5	1.28-1.34 (m)	1.18-1.24 (m)	1.21-1.27 (m)
5"	1.26-1.32 (m)	25.5	1.28-1.34 (m)	1.18-1.24 (m)	1.21-1.27 (m)
6"	1.26-1.32 (m)	25.5	1.28 (m)	1.18-1.24 (m)	1.21-1.27 (m)
7"	1.71-1.75 (m)	29.2	1.85-1.89 (m)	1.77-1.81 (m)	1.79-1.83 (m)
8"	2.04-2.05 (m)	58.5	3.12-3.15 (m)	2.58-2.60 (m)	3.03-3.05 (m)
9"	1.71-1.75 (m)	29.2	1.85-1.89 (m)	1.77-1.81 (m)	1.79-1.83 (m)
10"	1.26-1.32 (m)	25.5	1.28-1.34 (m)	1.18-1.24 (m)	1.21-1.27 (m)
11"	1.26-1.32 (m)	25.5	1.28-1.34 (m)	1.18-1.24 (m)	1.21-1.27 (m)
12"	1.26-1.32 (m)	25.5	1.28-1.34 (m)	1.18-1.24 (m)	1.21-1.27 (m)
13"	1.71-1.75 (m)	29.2	1.85-1.89 (m)	1.77-1.81 (m)	1.79-1.83 (m)

of potent compound 7. Further, it was evaluated by cell cycle analysis through flow-cytometry, reactive oxygen species and lactate dehydrogenase production. The caspase-9 and -3 activity were estimated by mitochondrial membrane potential (Abbas et al., 2021; Uddin et al., 2020).

4. CONCLUSION

Flavones and flavanones are two primary classes of Flavonoids, considered anti-oxidant compounds. In order to enhance their biological activity and checked structure-activity relationships, their Mannich bases were synthesized by Mannich reaction and evaluated for cancer activity. Three out of seven compounds exhibited more than 50% inhibition of cancer cell lines, and these compounds were evaluated for cytotoxic potential towards normal cell lines. MTT assay results exhibited that the cytotoxic profile of compound 7 against cancer cells is very appealing. The compound has proved its capability to produce toxic effects in cancerous cells by targeting mitochondria, inducing ROS, releasing lactate dehydrogenase, activating caspases, and causing cell cycle arrest in cancer cells. Therefore, it may be suggested that our compound has the potential in inducing apoptosis.

Table 3
Inhibitory concentrations of pure compounds and cisplatin in HeLa, MCF-7, and BHK-21 cells.

Codes	Cervical cancer cells (HeLa)	Breast cancer cells (MCF-7)	Baby hamster kidney cells (BHK–21)
	IC_{50} ±SEM (μM)/%age inhibition		
Compound 1	8.07 ± 0.21	42.0%	34.4 ± 1.34
Compound 2	8.09%	1.72%	-
Compound 3	11.8%	27.1%	-
Compound 4	31.4%	4.04%	-
Compound 5	28.8%	34.6%	-
Compound 6	14.6 ± 0.72	40.9%	22.1 ± 0.86
Compound 7	2.94 ± 0.13	29.7%	9.41 ± 0.25
Cisplatin	11.3 ± 0.78	6.2 ± 0.72	24.69 ± 0.37

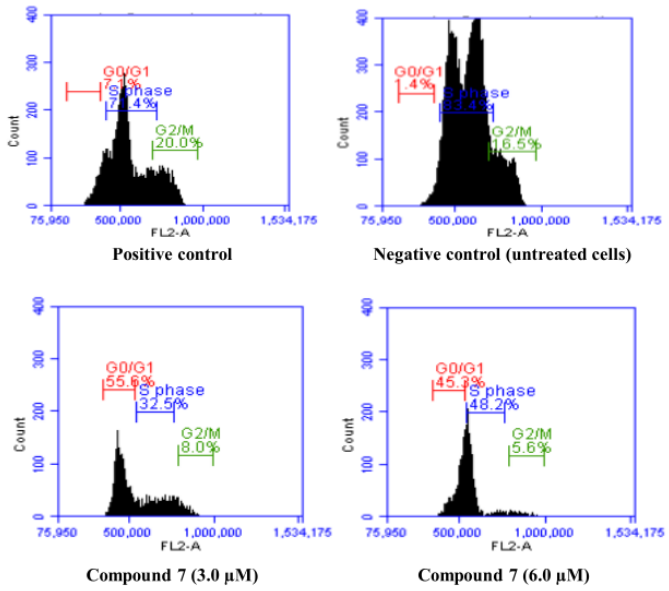


Figure 1. Propidium iodide staining with flow cytometry. G0/G1,S and G2/M stages in HeLa cells treated with cisplatin, untreated and compound 7.

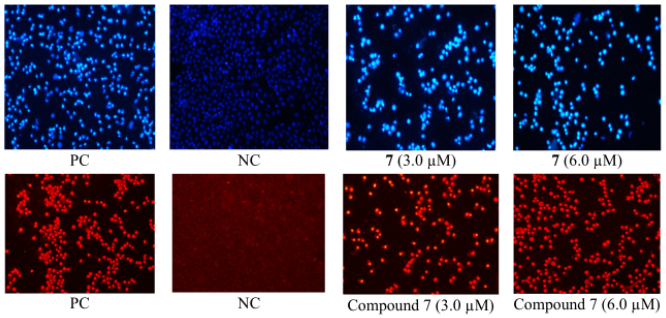


Figure 2. Induction of nuclear morphology after treatment with compound 7 and labelling with DAPI (upper image) and PI (lower image) in HeLa cells.

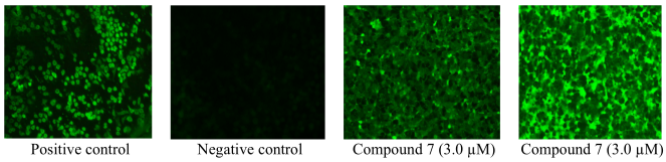


Figure 3. HeLa cells treated with compound 7, cisplatin, or 2',7'-dichlorofluorescin diacetate show oxidised DCF fluorescence. Photographed in HeLa cells with a fluorescent microscope.

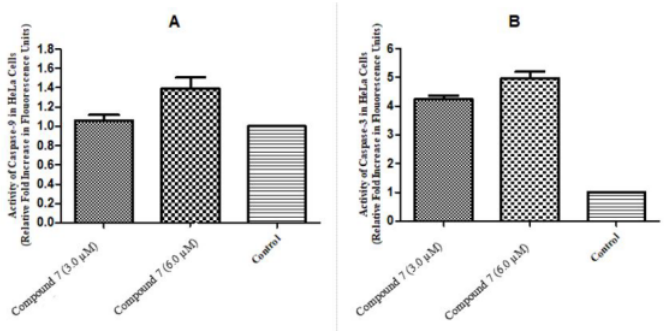


Figure 4. Compound 7 increases Caspase-9 and Caspase-3 activity in HeLa cells.

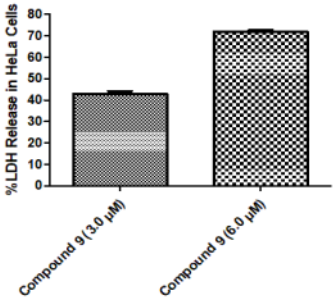


Figure 5. Compound 7 affects LDH release in HeLa cells.

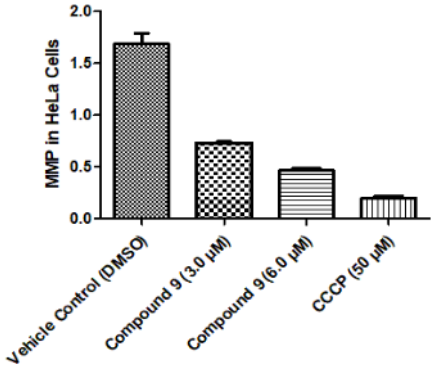


Figure 6. Compound 7 has an effect on the mitochondrial membrane potential in HeLa cells. Untreated cells retained a normal $\Delta\Psi m$, whereas treated cells exhibit a compromised $\Delta\Psi m$.

CONFLICTS OF INTEREST

In submitting or publishing this research, the authors claim no conflicts of interest.

ORCID

Huma Aslam Bhatti	0000-0002-8040-8916
Rubina Rubina	0000-0002-3808-9050
Faisal Rashid	0000-0003-0123-0505
Sumera Zaib	0000-0002-1894-6772
Jamshed Iqbal	0000-0002-8971-133X
Abdul Hameed	0000-0001-7498-1241

A. APPENDIX, SUPPLEMENTARY INFORMATION

Supplementary data to this article can be found online at https://doi.org/10.53365/nrfhh/141866.

AUTHOR CONTRIBUTIONS

HAB, - Research design, RR, SZ, FR - Data collection, RR, AH, JI - Data interpretation, RR, HAB - article writing HAB, JI, AH - Article revision, HAB, JI, AH - Article approval.

REFERENCES

Abbas, S., Rashid, F., Ulker, E., Zaib, S., Ayub, K., Ullah, S., Nadeem, M.A., Yousuf, S., Ludwig, R., Ali, S., Iqbal, J., 2021. Anticancer evaluation of a manganese complex on HeLa and MCF-7 cancer cells. Journal of Biomolecular and Structural Dynamics. 39, 1068–1081. https://doi.org/10.1080/07391102.2020.1726818

Armstrong, R.W., Combs, A.P., Tempest, P.A., Brown, S., Keating, T.A., 1996. Multiple-component condensation strategies for combinatorial library synthesis. Accounts of Chemical Research. 29(3), 123–131. https://doi.org/10.1021/ar9502083

Ashok, M., Holla, B., Poojary, B., 2007. Convenient one pot synthesis and antimicrobial evaluation of some new Mannich bases carrying 4-methylthiobenzyl moiety. European Journal of Medicinal Chemistry. 42(8), 1095–1101. https://doi.org/10.1016/j.ejmech.2007.01.015

Blagosklonny, M.V., 2005. Overcoming limitations of natural anticancer drugs by combining with artificial agents. Trends in Pharmacological Sciences. 26(2), 77–81.

Braca, A., Sortino, C., Politi, M., Morelli, Mendez, J., 2002. Antioxidant activity of flavonoids from Licania licaniaeflora. Journal of ethnopharmacology. 79(3), 379–381. https://doi.org/10.1016/S0378-8741(01)00413-5

Brodowska, K.M., 2017. Natural flavonoids: classification, potential role, and application of flavonoid analogues. European Journal of Biological Research. 7(2), 108–123. http://dx.doi.org/10.5281/zenodo.545778

Dua, R., Shrivastava, S., Sonwane, S.K., Srivastava, S.K., 2011. Pharmacological significance of synthetic heterocycles scaffold: a review. Advances in Biological Research. 5(3), 120–144.

Gordaliza, M., 2007. Natural products as leads to anticancer drugs. Clinical and Translational Oncology. 9(12), 767–776. https://doi.org/10.1007/s12094-007-0138-9

Haji, M., 2016. Multicomponent reactions: A simple and efficient route to heterocyclic phosphonates. Beilstein Journal of Organic Chemistry. 12, 1269–1269. https://dx.doi.org/10.3762/bjoc.12.121

Hanahan, D., Weinberg, R.A., 2011. Hallmarks of Cancer: The Next Generation. Cell. 144(5), 646–674. https://doi.org/10.1016/j.cell.2011.02.013

Hassanpour, S.H., Dehghani, M., 2017. Review of Cancer from Perspective of Molecular. Journal of Cancer Research and Practice. 4, 127–129.

Heng, H.H.Q., Stevens, J.B., Bremer, S.W., Ye, K.J., Liu, G., Ye, C.J., 2010. The Evolutionary Mechanism of Cancer. Journal of Cellular Biochemistry. 109(6), 1072–1084.

Joshi, S., Khosla, Tiwari, P., 2004. In vitro study of some medicinally important Mannich bases derived from antitubercular agent. Bioorganic & Medicinal Chemistry. 12(3), 571–576. https://doi.org/10.1016/j.bmc.2003.11.001

Kantlam, C., Murthy, M., Reddy, Y.N., 2015. Synthesis, Characterization and Antibacterial Activity of some Novel Mannich Derivatives of 3-(Benzofuran-2-Yl)-1-(4-Hydroxy-3-Methoxyphenyl) Prop-2-En-1-One. Journal of Pharmaceutical Research. 14(4), 90–93. https://dx.doi.org/10.4103/2231-4040.126983

Khan, N., Al-Daghri, N.M., Al-Ajlan, A., Alokail, M.S., 2014. The use of natural and derived sources of flavonoids and antioxidants in Saudi Arabia. Heart Disease. 8, 9–9. https://doi.org/10.15761/ifnm.1000109

Kim, S., 2015. New and emerging factors in tumorigenesis: an overview. Cancer Management and Research. 7(1), 225–239. https://doi.org/10.2147/cmar.s47797

Liaqat, M., Mahmud, T., Ashraf, M., Muddassar, M., Imran, M., Ahmad, Mitu, L., 2017. Synthesis, Characterization and Biological Activities of a Novel Mannich Base 2-[(3, 4-dimethoxyphenyl)(pyrrolidinyl) methyl] cyclohexanone and its Complexes with Cu (II), Ni (II), Co (II) and Fe (II) Ions. Revista de Chimie. 68(12), 2845–2849. https://doi.org/10.37358/RC.17.12.5991

Lin, G.J., Jiang, G.B., Xie, Y.Y., Huang, H.L., Liang, Z.H., Liu, Y.J., 2013. Cytotoxicity, apoptosis, cell cycle arrest, reactive oxygen species, mitochondrial membrane potential, and Western blotting analysis of ruthenium (II) complexes. JBIC Journal of Biological Inorganic Chemistry. 18(8), 873–882. https://doi.org/10.1007/s00775-013-1032-2

Liu, R., Zhao, B., Wang, D.E., Yao, T., Pang, L., Tu, Wang, J., 2012. Nitrogen-containing apigenin analogs: preparation and biological activity. Molecules. 17(12), 14748–14764. https://doi.org/10.3390/molecules171214748

Lopes, F., Capela, R., Gonçaves, J.O., Horton, P.N., Hursthouse, M.B., Iley, Moreira, R., 2004. Amidomethylation of amodiaquine: antimalarial N-Mannich base derivatives. Tetrahedron Letters(41), 7663–7666. https://doi.org/10.1016/j.tetlet.2004.08.093

Maccoss, M., Baillie, T.A., 2004. Organic chemistry in drug discovery. Science. 303(5665), 1810–1813. https://doi.org/10.1126/science.1096800

Malinka, W., Świątek, P., Filipek, B., Sapa, J., Jezierska, Koll, A., 2005. Synthesis, analgesic activity and computational study of new isothiazolopyridines of Mannich base type. Il Farmaco. 60, 961–968. https://doi.org/10.1016/j.farmac.2005.08.005

Miller, K.D., Siegel, R.L., Lin, C.C., Mariotto, A.B., Kramer, J.L., Rowland, J.H., Stein, K.D., Alteri, R., Jemal, A., 2016. Cancer treatment and survivorship statistics. CA: A Cancer Journal for Clinicians. 66(4), 271–289. https://doi.org/10.3322/caac.21565

Pandey, R.P., Gurung, R., Sohng, J.K., 2015. Dietary sources, bioavailability and biological activities of naringenin and its derivatives, N.M. Stacks, (Eds.), Apigenin and Naringenin: Natural Sources, Pharmacology and Role in Cancer Prevention. Nova Science Publishers, New York, pp. 151–172.

Rastogi, R.P., Singh, S.P., Hader, D.P., Sinha, R.P., 2010. Detection of reactive oxygen species (ROS) by the oxidant sensing probe 2',7'- dichlorodihydrofluorescein diacetate in the cyanobacterium Anabaena variabilis PCC 7937. Biochemical and Biophysical Research Communications. 397(3), 603–607. https://doi.org/10.1016/j.bbrc.2010.06.006

Ross, J., Kasum, C.M., 2002. Dietary flavonoids: bioavailability, metabolic effects, and safety. Annual review of Nutrition. 22, 19–34. https://doi.org/10.1146/annurev.nutr.22.111401.144957

Saini, N., Gahlawat, Lather, V., 2017. Flavonoids: A Nutraceutical and Its Role as Anti-inflammatory and Anticancer Agent, In: 1st (Eds.); S.K. Gahlawat, R.K. Salar, P. Siwach, J.S. Duhan, P. Kaur, et al., (Eds.), Plant Biotechnology: Recent Advancements and Developments. Springer, Singapore, pp. 255–270. https://doi.org/10.1007/978-981-10-4732-9_13

Sanchez, I., Gómez-Garibay, F., Taboada, Ruiz, B., 2000. Antiviral effect of flavonoids on the dengue virus. Phytotherapy Research. 14(2), 89–92. https://doi.org/10.1002/(SICI)1099-1573(200003)14:2<89::AID-PTR569>3.0.CO;2-C

Sánchez-Roque, Y., Ayora-Talavera, G., Rincón-Rosales, R., Gutiérrez-Miceli, F.A., Meza-Gordillo, R., Winkler, R., Ruiz-Valdiviezo., 2017. The flavonoid fraction from rhoeo discolor leaves acting as antiviral against influenza a virus. Records of Natural Products. 11, 532–546. http://doi.org/10.25135/rnp.68.17.01.017

Santos, E.L., Maia, B.H.L.S., Ferriani, A., Teixeira, S.D., 2017. Flavonoids: Classification, Biosynthesis and Chemical Ecology, and others, (Eds.), Flavonoids - From Biosynthesis to Human Health. InTech.

Scifo, C., Cardile, V., Russo, A., Consoli, R., Vancheri, C., Capasso, F., Vanella, A., Renis, M., 2004. Resveratrol and propolis as necrosis or apoptosis inducers in human prostate carcinoma cells. Oncology Research. 14(9), 415–426. https://doi.org/10.3727/0965040041791437

Shang, S., Tan, D.S., 2005. Advancing chemistry and biology through diversity-oriented synthesis of natural product-like libraries. Current Opinion in Chemical Biology. 9(3), 248–258. https://doi.org/10.1016/j.cbpa.2005.03.006

Sujith, K., Rao, J.N., Shetty, Kalluraya, B., 2009. Regioselective reaction: synthesis and pharmacological study of Mannich bases containing ibuprofen moiety. European Journal of Medicinal Chemistry. 44(9), 3697–3702. https://doi.org/10.1016/j.ejmech.2009.03.044

Tanaka, T., Higa, S., Hirano, T., Kotani, M., Matsumoto, M., Fujita, Kawase, I., 2003. Flavonoids as potential anti-allergic substances. Current Medicinal Chemistry-Anti-Inflammatory & Anti-Allergy Agents. 2(1), 57–65. https://doi.org/10.2174/1568014033355790

Uddin, N., Rashid, F., Ali, S., Tirmizi, S.A., Ahmad, I., Zaib, S., Zubair, M., Diaconescu, P.L., Tahir, M.N., Iqbal, J., Haider, A., 2020. Synthesis, characterization, and anticancer activity of Schiff bases. Journal of Biomolecular Structure and Dynamics. 38, 3246–3259. https://doi.org/10.1080/07391102.2019.1654924

Yeh, B.J., Lim, W.A., 2007. Synthetic biology: lessons from the history of synthetic organic chemistry. Nature Chemical Biology. 3(9), 521–525.

Zhang, X., Li, H., Zhang, H., Liu, Y., Huo, L., Jia, Zhang, W., 2017. Inhibition of transmembrane member 16A calcium-activated chloride channels by natural flavonoids contributes to flavonoid anticancer effects. British Journal of Pharmacology. 174(14), 2334–2345. https://doi.org/10.1111/bph.13841