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Research Article

Synthesis, Biological Evaluation, and Docking of Dihydropyrazole Sulfonamide Containing 2-hydroxyphenyl Moiety: A Series of Novel MMP-2 Inhibitors

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In this study, we synthesized a series of dihydropyrazole sulfonamide derivatives containing 2-hydroxyphenyl moiety as antitumor agents to target the matrix metalloproteinase-2 (MMP-2). All of the synthesized compounds were examined by bioactivity assays, in which compound 4c turned out as a potential antagonist of MMP-2 along with potent anticancer activity against four tumor cell lines. Structure–activity relationship analysis was also performed to examine how structural changes impacted the bioactivity. Suggested to be caused by the induction of apoptosis, the antitumor mechanism of 4c was further confirmed by PI combining with annexin V-FITC staining assay using flow cytometry analysis. These new findings along with molecular docking observations suggested that compound 4c could be developed as a potential anticancer agent.

Key words: anticancer, dihydropyrazole sulfonamide, extracellular matrix enzyme, flow cytometry analysis, MMP-2

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The matrix metalloproteinases (MMPs) represent a superfamily of zinc-dependent enzymes functioning as metalloproteases, whose proteolytic activity on the substrates of extracellular matrix (ECM) is crucial for physiological processes of tissue remodeling and repairing, homeostatic regulation, innate immunity control, and so on (1–3). The proteases are also capable to cleave non-matrix substrates, including growth factors, peptidase inhibitors, cytokines, receptors, adhesion molecules, clotting factors, and other proteases, and hence play a pleiotropic role in diverse bioprocesses (4). In normal circumstances, MMPs are tightly regulated and controlled in activities, and atypical alterations may lead to a range of pathologies; for example, aberrant increase gives rise to several diseases such as osteoarthritis (5), rheumatoid arthritis (6), periodontal disease (7), multiple sclerosis (8), and tumor metastasis (9). In particular, pathological level of MMPs has a close link with cancer in virtually all aspects of cancer progression and metastasis, although the mechanism is not fully understood yet. Still, MMPs have proven to be potential antitumor target, and their inhibitors possess the potency to serve as effective anticancer agents (10). For the last two decades or so, many research studies within academia and industries have been focused on the design and screening for potent MMPs inhibitors (11). In general, the MMPs inhibitors can be divided into two types according to whether they are binding to the activity site S1' cavities or chelating with the zinc ion (12,13). Zinc-binding groups (ZBGs) are essential to form strong co-ordination bond with the zinc cation, and by far, various groups have been validated as ZBGs including phenol hydroxy, secondary amine, amide, imine, imidazole, carboxylate, aminocarboxylate, sulphydryl, hydroxamate, phosphonate, and phosphinate moieties (14–18). However, although many efforts have been made, they appeared to be insufficient due to the constraints of toxicity and dose-limiting efficacy which impeded the clinical use of these inhibitors (19). Thus, there remains a considerable need for the optimization of oral absorption, drug processes, and action duration while maintaining potency and selectivity. To address these shortfalls, new MMPs inhibitor templates should be elaborated and applied into the design.

In our previous work, compounds bearing the benzenesulfonate sulfonamide skeleton were synthesized and investigated for their bioactivities (20). To our delight, some showed potent activity against cancer cells and high safety to non-cancer cells. The aforementioned progress laid the foundation and encouraged us to carry out further study. From the previous docking mode of the most potent compound, benzenesulfonamide and appropriate stereo-space are found to be favorable, along with alteration in enzyme-active site caused by the binding. Our recent work focused on the design of new inhibitors on the basis of

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previous study, and the structure of dihydropyrazole was employed as it is less flexible than the -C-N- structure, offering more preferable stereo-space. Meanwhile, the benzenesulfonamide was retained as zinc-binding group and the result turned out to support our assumption. Based on the results, we concluded that all the newly synthesized compounds showed enhanced potential against different tumor cell lines and MMP-2 enzyme.

Results and Discussion

Chemistry

Scheme 1 illustrates the general synthetic route to obtain the target compounds (**4a**–**4y**). The starting chalcones (**3a**–**3y**) were directly condensed by equivalent salicylic aldehydes (**1a**–**1c**) and acetophenones (**2a**–**2g**). Catalyzed by 40% potassium hydroxide, the Claisen–Schmidt reactions were obtained and gave precipitates in ethanol cooled to the temperature of 0 °C. The precipitates were then filtered and washed by cold ethanol, and purified chalcones (**3a**–**3y**) were obtained. Concomitantly, target chalcones were dissolved in ethanol with 4-sulfamoylphenyl hydrazine hydrochloride, whereafter several drops of acetic acid were added and the mixture was heated to reflux to furnish compounds (**4a**–**4y**). All the compounds gave satisfactory analytical and spectroscopic data, and 23 compounds (**4a**–**4w**) were reported for the first time. Elemental analyses, ^{1}H NMR, and ESI-MS spectra were consistent with the assigned structures.

Crystal structures of compound 4x

Through recrystallization, the single crystal structure of compound **4x** was obtained and subsequently tested by X-ray diffraction analysis. The perspective view of compound **4x** with atomic labeling system is presented in Figure 1, and also the data are listed on Table S1.

Biological activity

MMP-2 inhibitory activity

All the synthesized compounds (**4a**–**4y**) were evaluated for their *in vitro* bioactivity against the MMP-2 enzyme inhibition compared to the control group CMT-1. The results are summarized on Table 1 and indicate that compound **4e** has the same effects as the positive control CMT-1 with the IC_{50} of 1.81 and 1.13 μM, respectively, while compound **4c** has

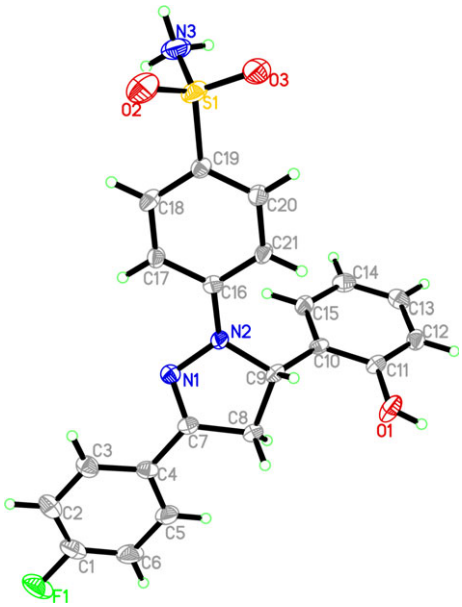
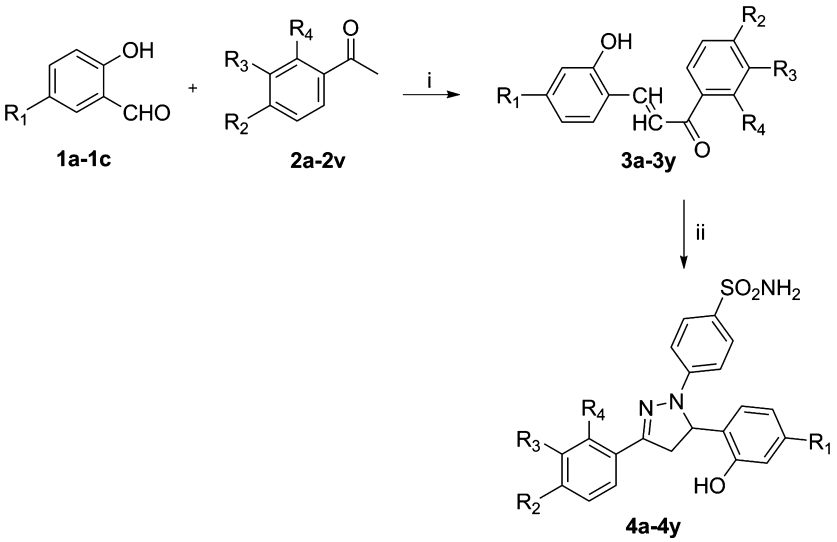


Figure 1: Crystal structure diagram of compound **4x**.



Scheme 1: General synthesis of derivatives (**4a**–**4y**). Reagents and conditions: (i) 40% aqueous potassium hydroxide solution, ethanol, ice bath; (ii) 4-sulfamoylphenyl hydrazine hydrochloride, glacial acetic acid, reflux, 6–8 h.

Table 1: Antiproliferation and enzyme inhibitory activities (IC_{50}[a], μM) of target compounds (**4a**–**4y**)

Compounds	R_1	R_2	R_3	R_4	IC_{50}[a] (μM)				
					A549	MCF-7	Hela	HepG2	MMP-2[b]
4a	H	H	H	H	3.97	2.68	1.70	4.56	9.83
4b	H	H	H	Cl	12.0	5.96	7.45	2.31	11.51
4c	H	OCH_2CH_3	H	H	1.93	4.37	3.17	4.21	0.33
4d	H	Br	H	H	2.88	6.31	4.68	7.05	18.76
4e	H	H	OCH_3	H	5.24	4.27	6.63	5.51	1.81
4f	H	CH_3	CH_3	H	6.18	5.30	8.64	9.54	4.65
4g	H	Cl	Cl	H	13.32	5.69	12.15	10.02	12.87
4h	Cl	F	H	H	6.49	3.14	4.51	7.21	8.83
4i	Cl	H	H	Cl	7.35	1.67	8.13	4.21	16.43
4j	Cl	H	H	H	19.4	1.85	16.52	9.64	13.54
4k	Cl	Cl	H	H	5.87	3.35	5.99	4.45	11.28
4l	Cl	OCH_3	H	H	7.39	1.87	8.53	8.73	4.62
4m	Cl	Br	H	H	14.03	1.62	9.87	6.77	18.90
4n	Cl	Cl	Cl	H	24.67	2.45	11.74	5.20	16.49
4o	Cl	OCH_2CH_3	H	H	9.52	22.94	10.05	11.38	3.84
4p	Cl	H	OCH_3	H	4.12	6.95	5.13	6.78	2.13
4q	Br	H	H	H	14.03	2.39	7.21	10.03	12.78
4r	Br	H	OCH_3	H	7.39	2.40	6.09	8.59	3.23
4s	Br	F	H	H	24.67	3.73	3.54	7.66	17.25
4t	Br	H	H	Cl	9.51	2.40	6.89	4.13	14.96
4u	Br	Cl	H	H	2.25	3.73	1.74	1.01	15.84
4v	Br	Br	H	H	4.12	2.40	4.95	5.69	8.73
4w	Br	Cl	Cl	H	5.24	13.96	14.98	4.51	16.24
4x	H	F	H	H	36.8	18.5	7.61	7.05	24.66
4y	H	OCH_3	H	H	8.93	12.6	7.85	11.51	1.83
Gefitinib -		–	–	–	2.83	6.76	1.43	–	–
Celecoxib -		–	–	–	2.26	6.89	7.61	0.73	–
CMT-1 -		–	–	–	–	–	–	–	1.13

[a]Values are the average of three independent experiments run in triplicate. Variation was generally 5–10%.
[b]Human recombinant enzymes, by the esterase assay (4-nitrophenylacetate as substrate).

stronger MMP-2 enzyme-suppressing capability than that of the positive control CMT-1, possessing IC_{50} of 0.33 μM. By analyzing the relationship between the structure and activity of the compounds, it can be inferred that the changing of substituent salicylaldehyde exhibited less effect on SAR, whereas the maximum impacts were observed from the substituents on the benzene ring of acetophenone. In details, when the substituents on the *para*-position of the benzene ring are potent electron-donating groups rather than electron-withdrawing groups, the compounds gained better activity, for **4c** (IC_{50} = 0.33 μM) > **4a** (IC_{50} = 9.8 μM) > **4d** (IC_{50} = 18.76 μM); the substituents on *meta*-position also mattered, for **4p** (IC_{50} = 2.13 μM) > **4j** (IC_{50} = 13.54 μM). However, substituent salicylaldehydes had minor effect on the inhibitory variations of these compounds, indicated by the order **4c** (IC_{50} = 0.33 μM) < **4e** (IC_{50} = 1.81 μM) < **4p** (IC_{50} = 2.13 μM) < **4r** (IC_{50} = 3.23 μM).

In comparison, we found that these compounds, with electron-donating substituents on the benzene ring (such as CH_3, OCH_3), exhibited more potent anticancer activities than those having bulky or electron-withdrawing group (such as F). From the aforementioned analysis, it could be concluded that the electron-donating substituents were more ideal groups than electron-withdrawing groups in enhancing pharmaceutical potency on the whole.

Antiproliferation assay

All the synthesized compounds (**4a**–**4y**) were evaluated for their antiproliferative activities against different tumor lines of MCF-7, HepG2, Hela, and A549, whereas two classical clinical drugs gefitinib and celecoxib were used as positive control. The bioactive data summarized on Table 1 demonstrated that all the compounds possessed moderate to good restraint abilities. Against MCF-7 cell line,several compounds were comparable to the positive group drugs, such as **4b**, **4d**, **4f**, **4g** and **4p**, while inspiringly most of the rest compounds were more potent than the control groups. Also against the other cancer cell lines, most compounds exhibited impressive potency. Taking all the results into consideration, compounds **4a**, **4c** and **4u** revealed comprehensive inhibition abilities, and for all, the corresponding IC_{50} was below 5 μM.

Cytotoxicity

All the target compounds were evaluated for their cytotoxicity against human kidney epithelial cell 293T with the

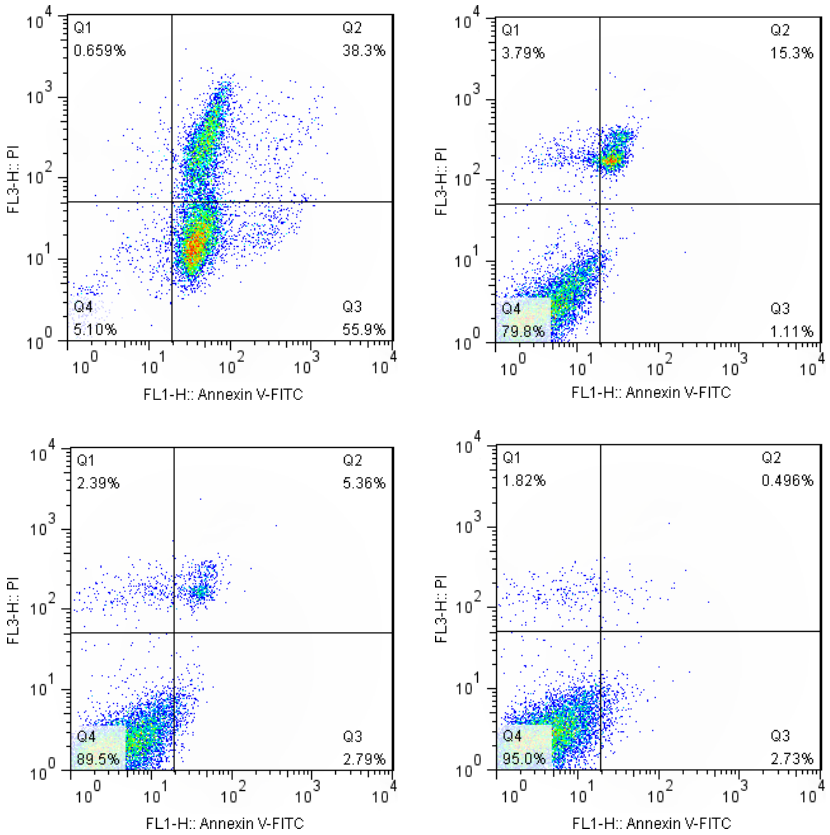


Figure 2: Compound **4c** induced apoptosis in Hela cells with the density of 32, 8, 2 and 0 μM. Hela cells were treated with compounds for 24 h. Values represent the mean ± SD, $n = 3$. $p < 0.05$ versus control. The percentage of cells in each part was indicated.

median cytotoxic concentration (CC_{50}) data of tested compounds by the MTT assay. These compounds were tested at multiple doses to study the viability of 293T cells, and as showed on Table S2, all the compounds demonstrated low cytotoxic activities *in vitro* against human kidney epithelial cell 293T.

Apoptosis assay

To verify whether the inhibition of cell growth of Hela was related to cell apoptosis, apoptosis of Hela cells induced by compound **4c** was determined using flow cytometry. The result is shown in Figure 2. According to the data annotated, the percentage of apoptotic cells elevated directly and markedly increased in a dose-dependent manner The percentages of cell apoptosis 3.26%, 8.35%, 16.4% and 94.2% were responding to the concentration of compound **4c** 0, 2, 8 and 32 μM.

Molecular docking

Docking study was performed to fit these compounds into the active site of the matrix metalloproteinases MMP-2 (PDB code: 1QIB). The probable binding mode of compound **4c** which showed the most potent enzymatic inhibition is presented in Figures S1 and S2. This model revealed that the amino acid residues TYR155, PHE157, and PHE180 located in the binding cavity are important in binding with compound **4c**. Two hydrogen bonds and two pi–pi interactions could be found in the 2D model, while the 3D model revealed that two co-ordination bonds were also formed between zinc cation and **4c**. The docking results suggested that the sulfanilamide and salicylaldehyde backbone are of vital importance, while the substitute acetophenones provide the compounds with admirable steric stabilization.

Conclusion

To summarize, on the basis of previous study, a series of novel MMP-2 inhibitors (**4a**–**4y**) bearing sulfonamide skeleton have been synthesized and examined for their biological activities. The results suggested that these inhibitors possess improved activities, exhibiting moderate to potent antiproliferative potency against MCF-7, Hela, HepG2, and A549 cells and enzyme MMP-2. Among them, **4a**, **4c** and **4u** showed the most potent inhibitory activities against all the cell lines with IC_{50} values smaller than 5 μM. Besides, compound **4c** inhibited the MMP-2 with IC_{50} of 0.33 μM. The probable binding models were obtained by docking simulation, suggesting that the sulfanilamide and salicylal-

dehyde backbone are favorable for the zinc-chelating bonds and hydrogen bonds which provide considerable steric binding stabilization.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Experimental details, NMR, MS and elemental analyses data for the synthesized compounds.

Figure S1. Molecular docking 2D modeling of compound **4c** with MMP-2.

Figure S2. Molecular docking 3D modeling of compound **4c** with the MMP-2 binding site: for clarity, only interacting residues are displayed.

Table S1. Crystal data for compound **4x**.

Table S2. The median cytotoxic concentration (CC_{50}) data of all compounds (**4a**–**4y**).