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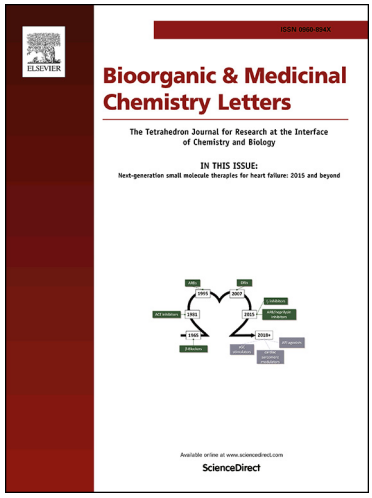

Dihydropyrazothiazole derivatives as potential MMP-2/MMP-8 inhibitors for cancer therapy

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PII: S0960-894X(18)30394-9
DOI: https://doi.org/10.1016/j.bmcl.2018.05.004
Reference: BMCL 25821

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 11 February 2018
Revised Date: 17 April 2018
Accepted Date: 2 May 2018

Please cite this article as: Wang, Z-C., Shen, F-Q., Yang, M-R., Xia-You, L., Chen, L-Z., Zhu, H-L., Lu, Y-D., Kong, F-L., Wang, M-H., Dihydropyrazothiazole derivatives as potential MMP-2/MMP-8 inhibitors for cancer therapy, *Bioorganic & Medicinal Chemistry Letters* (2018), doi: https://doi.org/10.1016/j.bmcl.2018.05.004

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Dihydropyrazothiazole derivatives as potential MMP-2/MMP-8 inhibitors for cancer therapy

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Abstract

MMP-2/MMP-8 is established as one of the most important metalloenzymes for targeting cancer. A series of dihydropyrazothiazole derivatives **(E1-E18**) bearing a salicylaldehyde group linked to Pyrazole ring were designed, synthesized, and evaluated for their pharmacological activity as MMP-2/MMP-8 inhibitors. Among them, compound **E17** exhibited most potent inhibitory activity (IC_{50} =2.80 μM for MMP-2 and IC_{50}=5.6 μM for MMP-8), compared to the positive drug CMT-1 (IC_{50} =1.29 μM). Compounds (**E1-E18**) were scrutinized by CoMFA and CoMSIA techniques of Three-dimensional quant. structure-activity relationship (3D-QSAR), as well as a docking simulation. Moreover, treatment with compound **E4** could induce MCF-7 cell apoptosis. Overall, the biological profile of **E1-E18** may provide a research basis for the development of new agents against cancer.

Keywords:

Dihydropyrazothiazole

MMP-2/MMP-8

Synthesis

3D-QSAR

Docking simulation

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Malignant tumor, also known as cancer, is a common and frequent malignant disease that seriously threatens human health. Its incidence is the second highest in the global cancer prevalence rate[1]. Although much efforts has been made in developing new drugs to cure cancer, improving survival, the number of deaths caused by cancer is still very high[2], due to lack of selectivity and high cytotoxicity in traditional medicine, metastasis of cancer cell, the diversity of cancer and drug resistance [3]. Therefore, it is imminent for researchers to find selective inhibitory anticancer drugs with high efficiency and low toxicity.

Matrix metalloproteinases (MMPs) are a family of extracellular zinc and calcium-dependent neutral endopeptidases structurally and functionally related, playing a crucial role in degradation of extracellular matrix components, tissue remodeling and the pathogenesis of major diseases[4]. MMPs are involved in a wide range of physiological processes, including ovulation, embryonic development, angiogenesis, cellular variation, and wound healing[5]. Furthermore, they also play a key role in degrading the major components of the extracellular matrix, inflammation, tumor cells proliferation, and some other typical diseases[6]. MMPs play a crucial role in the remodeling and repair of physiological tissues, but their overexpression has led to a wide variety of diseases[7], such as cancer[8], arthritis[9], osteoporosis[10], and cirrhosis[11]. Therefore, inhibiting MMPs is an attractive approach which can treat multitude of diseases. Much important progress has been made in the mechanism of MMPs worldwide from the early 90s of the last century[12], For example, many inhibitors of MMP-2 have been proved to prevent cancer tumor growth,[13] some of which had reached an advanced clinical trial. Representative examples include **1-5** had been tested against cancers;[14] MMP inhibitors **6** (cipemastat)[15] and **7** (illomastat)[16] were tried in the clinics for inflammation (Chart1).

Unfortunately, however, there had been no clinically useful inhibitor to be demonstrated until now. This is mainly because the serious side effects, like musculoskeletal syndrome, which would fail the clinical trials[17]. Meanwhile, there are some other reasons, such as MMPs involving in promotion of some pathological process, converse in other pathological process[18]. In addition, previous attempts to study targeting MMPs ended in failure due to a lack of understanding of the role of MMPs in physiology and pathology[19].

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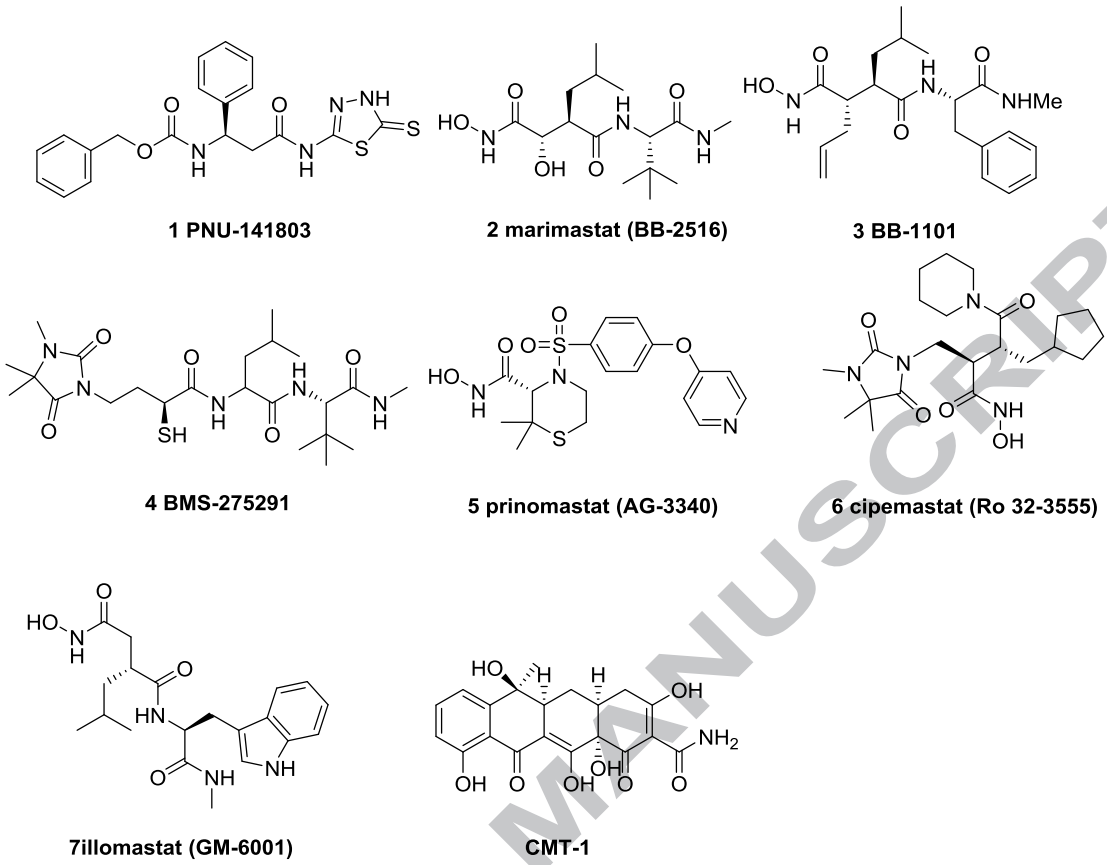

Chart1

On the other hand, all of the first generation and most of the second generation matrix metalloproteinase inhibitors (MMPIs) failed in application and promotion due to lack of specificity[20]. Therefore, at present, selective inhibitors without zinc ions or phosphate groups are considered as both targets and antitargets for cancer therapy[21]. Specifically, MMP-2, MMP-3, MMP-8, MMP-9, MMP-12 and MMP-14 are considered as antitargets whereas MMP-2/MMP-8, the focus of our study, is taken into account as a target and play a role in blocking the proliferation of tumor cells[22]. In the past few decades, highly efficient inhibitors of MMP has opened up a new horizon for researchers[23].

So far, tetracycline and its derivatives were mostly found as natural inhibitiors of MMPs. With the in-depth study, tetracyclines or structural modifications of tetracycline derivatives can selectively inhibit MMPs[24], like structurally modified compound **CMT-1**. Based on the reported three-dimensional crystal structure information of MMPs and their small molecule ligand complexes, we use computer-aided drug design technology to screen a series of dihydropyrazothiazole derivatives **E1-E18** as potential MMP-2 inhibitors according to the size and shape of

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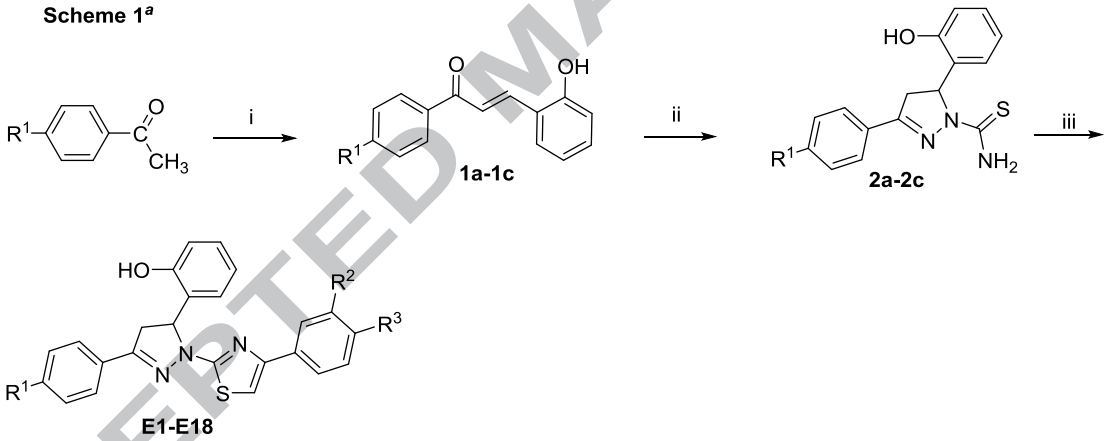
the receptor binding pocket, the binding site of the binding region and the transformable space of non-binding region. This study may contribute to discover potential and selective MMP-2 inhibitors for the treatment of cancer.

All of the novel dihydropyrazothiazoles containing salicylaldehyde moieties followed the synthetic pathway was depicted in Scheme 1[a]. The starting diverse substituted chalcones (1a-1c) were synthesized by the cross-condensation reaction of salicylaldehyde with the substituted acetophenone in the presence of 40% KOH solution, acting as catalyst, in ethanol, then cyclized by thiosemicarbazide at room temperature to afford the formation of Salicylaldehyde dihydropyrazolamide (**2a-2c**). Subsequently, Compounds (**2a-2c**) and 2-bromo-1-phenyl were dissolved in dimethylformamide (DMF) to obtain target compounds (**E1-E18**). All of the target compounds are reported for the first time, and gave satisfactory analytical and spectroscopic data ^{1}H NMR and ESI-MS, which are in full accordance with their depicted structures.

Scheme 1[a]



[a] Reagents and conditions: (i) 1.0 equiv. 2-hydroxybenzaldehyde, EtOH, KOH, H_2O rt, 4 h; (ii) 1.0 equiv. thiosemicarbazide, EtOH, AcOH, rt, 8 h; (iii) 1.0 equiv. 2-Bromoacetophenone, DMF, rt, 6 h.

All target compounds (**E1-E18**) were evaluated for their antiproliferatve activities *in vitro* against three cancer cell lines, including MCF-7, HepG2, HeLa, compared with the positive contrast drugs **Gefitinib** and **Celecoxib**. As shown in Table 1, MCF-7 was effectively suppressed by most of the target compounds, which showed better anti-proliferative activities than positive contrast drugs, especially **E4** (IC_{50} up to 1 μM).

Generally, the halogen substituent could enhance anti-cancer activity of synthesized compounds, while the strong electron-donating group failed. To be specific, when R^1, R^3 is substituted by F, Br, respectively, they showed more potent anti-proliferative activity on MCF-7 than other substituents. In order to verify our research results more deeply, compounds (**E1-E18**) were evaluated for their inhibitory activities on MMP-2/MMP-8 in our further study, with positive control drug **CMT-1**. The results

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were compared with **CMT-1** under identical conditions, which showed that the majority of the synthesized compounds exhibited excellent inhibiting MMP-2 activities displaying IC_{50} values between 2.8 and 58.8 μM, but weak to MMP-8. The following structure-activity relationship (SAR) studies were performed by modification of the parent compound to determine how the substituents of the subunits affect the MMP-2 inhibitory activities. When substituents R^2, R^3 remained unchanged, selection of different substituents R^1 against MMP-2 indicated strong electron-donating groups are preferable than electron-withdrawing groups. For instance, **E17** (IC_{50}=2.80 μM) > **E11** (IC_{50}=6.70 μM). R_1, conversely, was kept unaltered, alteration of R^2, R^3 showed that electron-donating groups could contribute to the increase of anti-cancer activity. Furthermore, compounds **E1-E18** exhibited moderate inhibitory activity against MMP-2 but failed to achieve the effects of positive control, even the most effective compound **E8** could just close to the half effect of **CMT-1**. After mentioned analysis, it could be concluded that dihydropyrazothiazole derivatives acted as MMP-2 inhibitors for cancer therapy.

Table 1 Data from *in vitro* anti-proliferative and MMP-2/MMP-8 inhibitory activities (IC_{50}, μM) of target compounds (**E1-E18**) (μM)

Compounds	**R^1**	**R^2**	**R^3**	$IC_{50}{}^a$ (μM)				
				MCF-7	HepG2	Hela	MMP-2	MMP-8
E1	H	H	H	2.08	9.90	5.05	21.64	62.5
E2	H	H	Br	2.14	17.58	4.88	34.76	75.6
E3	H	Br	H	1.07	21.68	5.51	28.33	86.5
E4	H	H	OCH_3	1.03	11.94	8.64	11.23	48.9
E5	H	OCH_3	H	5.3	10.87	2.73	8.31	35.8
E6	H	H	CF_3	3.5	8.85	2.25	48.16	>100
E7	F	H		3.24	25.00	13.14	9.65	39.7
E8	F	H	Br	1.47	15.04	2.70	12.32	48.8
E9	F	Br	H	8.01	18.41	10.85	16.24	60.1
E10	F	H	OCH_3	4.56	18.22	16.97	10.81	28.2
E11	F	OCH_3	H	4.01	17.75	9.73	6.70	36.4
E12	F	H	CF_3	2.89	10.49	11.65	58.43	100.1
E13	OCH_3	H	H	2.39	23.32	18.15	12.86	30.5
E14	OCH_3	H	Br	20.14	9.52	28.1	16.87	36.8
E15	OCH_3	Br	H	4.42	7.55	13.02	13.96	48.1
E16	OCH_3	H	OCH_3	3.17	15.28	16.31	11.72	65.2
E17	OCH_3	OCH_3	H	3.72	12.55	13.70	2.80	25.6
E18	OCH_3	H	CF_3	11.13	12.00	29.26	58.8	>100
Gefitinib	-	-	-	6.77	-	1.48	-	
Celecoxib	-	-	-	6.96	0.78	7.55	-	
CMT-1	-	-	-	-	-	-	1.29	21.9

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[a] Values are the average of three independent experiments run in triplicate. Variation was generally 5–10%.

The method of compound **E4** on MCF-7 apoptosis was studied by Annexin V-FITC/PI double staining. MCF-7 cells were treated with different concentrations (0 μM, 2 μM, 8 μM and 32 μM) of **E4** for 48 h. As illustrated in Fig. 1, the percentage of apoptotic cells (7.87%, 26.84%, 46.14% and 63.19%) increased in a dose-dependent manner, corresponding to concentrations of compound **E4** on 0 μM, 2 μM, 8 μM and 32 μM, respectively. In conclusion, compound **E4** could effectively induce MCF-7 cells apoptosis in a dose-dependent manner.

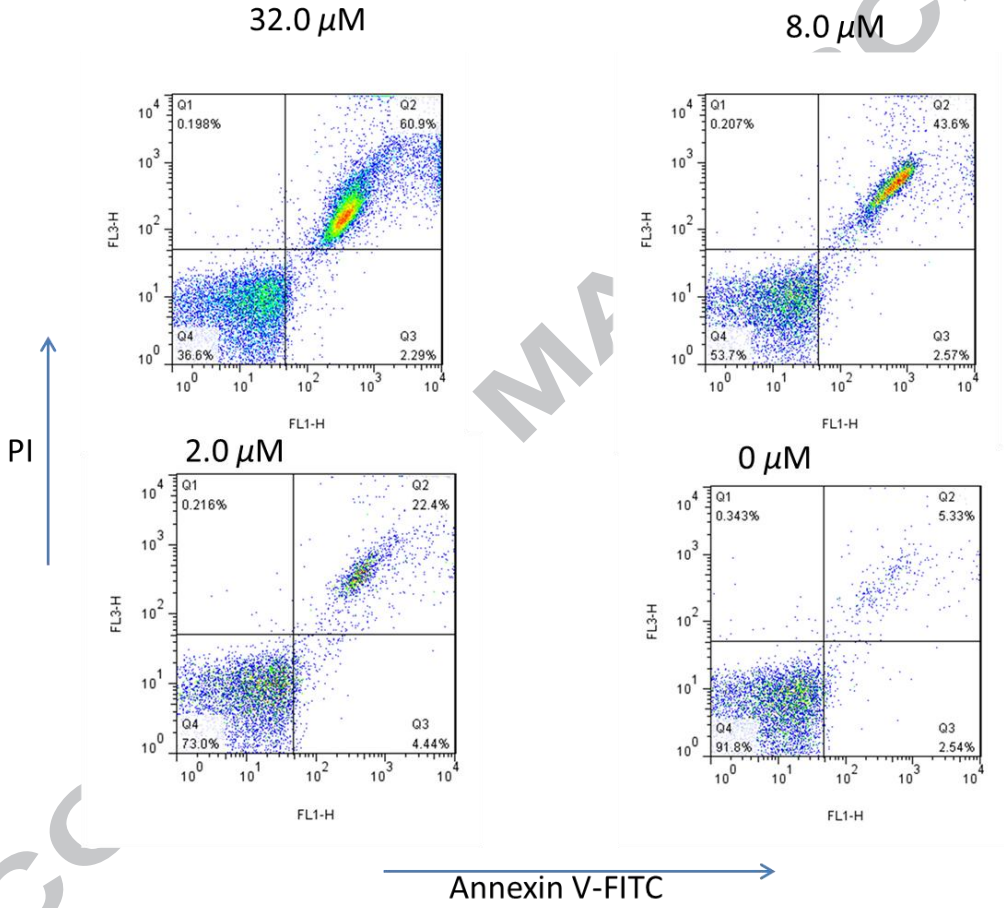

Fig. 1. MCF-7 cells treated with 0, 2, 8 and 32 μM 4d for 48 h were collected and analysed. Images are representative of three independent experiments.

Compounds **E1-E18** were evaluated for their toxicity against human kidney epithelial cell 293T/LO2, comparing with positive control drug **Celecoxib**, and the median cytotoxic concentration (CC_{50}) data (Table 2) of tested compounds was achieved using the MTT assay. As shown in **Table 2**, some of the target compounds exhibited less cytotoxicity against 293T/LO2 than the positive control drug. Hence, we were inspired by the discovery and conducted further investigation.

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Table 2 The median cytotoxic concentration (CC_{50}) data of compounds **E1-E18** against 293T/LO2

Compounds	CC_{50}, μmol[a]		Compounds	CC_{50}, μmol[a]	
	293T	LO2		293T	LO2
E1	55.10	88.3	**E11**	39.15	43.8
E2	52.77	65.2	**E12**	34.78	66.1
E3	41.77	42.3	**E13**	64.07	75.2
E4	57.12	72.4	**E14**	71.94	>100
E5	46.63	57.9	**E15**	24.50	68.7
E6	25.38	42.8	**E16**	63.04	49.7
E7	50.73	85.6	**E17**	35.22	56.2
E8	52.01	48.2	**E18**	45.43	45.6
E9	70.09	59.4	**Celecoxib**	55.83	78.2
E10	59.42	85.6			

[a] Values are the average of three independent experiments run in triplicate. Variation was generally 5-10%.

From preliminary virtual and *in vitro* MMP-2 inhibitory activities of salicylidene dihydropyrazole thiazide derivatives screening results showed that, the IC_{50} values of **E15**, **E16** against MMP-2 were 13.96 and 11.72 μM, respectively. As shown in the docking model of **E16** and MMP-2 (Fig. 2), **E16** had functional groups complexing with Zn^{2+}. Besides, N-grafted thiazole skeleton in the dihydropyrazole ring could penetrate into the S1 binding pocket, and *p*-methoxybenzene ring grafted at the C-3 position could penetrate into the S2 'and S3' binding cavities. Therefore, compound **E16** fitted each pocket cavity of MMP-2 well and stabilized its binding conformation. Meanwhile, a strong π bond was formed between the phenyl ring at the 2-position of thiazole and amino acid PHE. Thus it can be speculated that salicylaldehyde containing dihydropyrazole thiazole derivatives can fit into each pocket of MMP-2 well. We retained the dihydropyrazole thiazole derivative skeleton with thiazole at the N-1 position, followed by further structure optimization, and explored the possibility and the structure-activity relationship of this compound as a lead compound of MMP-2 inhibitor.

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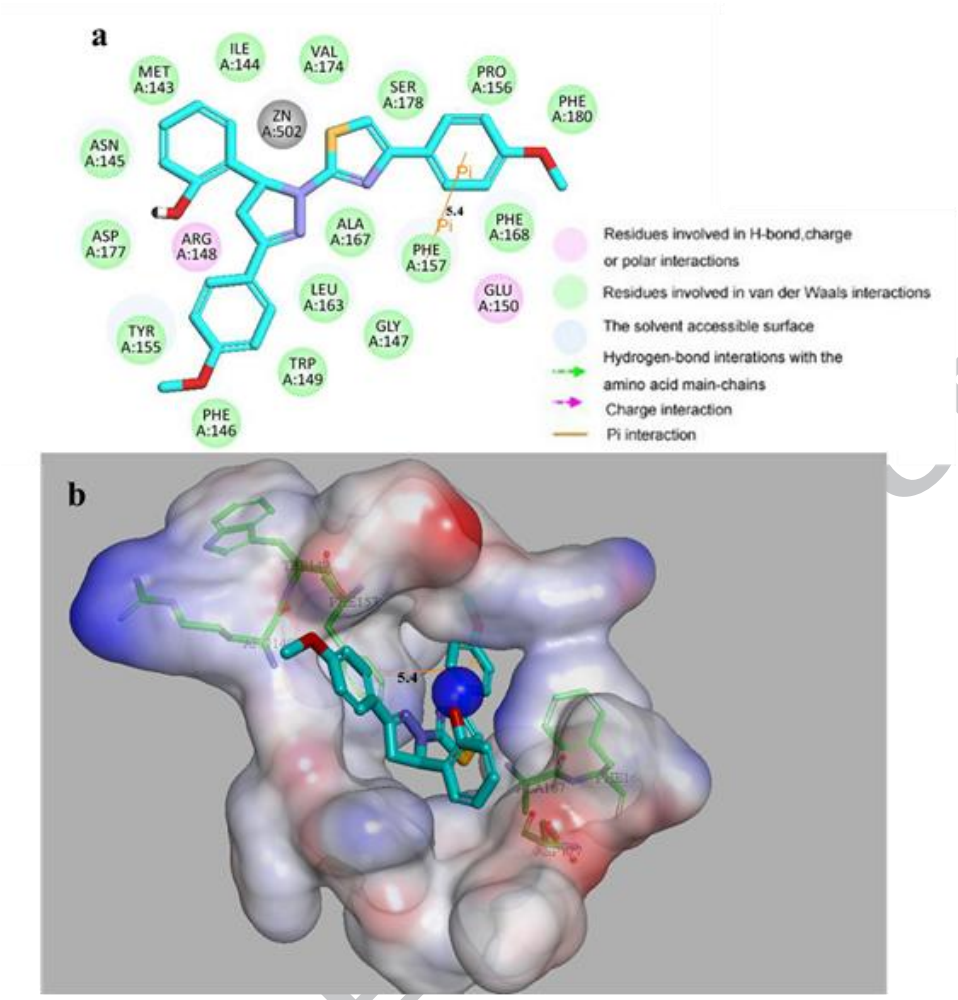

Fig. 2 (a) Interactions between compound **E16** and MMP-2 in a two-dimensional plan (b) Interactions between compound **E16** and MMP-2 in a three dimentional stereogram

In order to acquire a systematic and visual SAR profile on dihydropyrazothiazole derivatives bearing a salicylaldehyde group as MMP-2 inhibitors and to explore more clues which led to further optimization of inhibitory molecular structure, 3D-QSAR model of compounds **E1-E18** was built, and hence we can choose activity conformation of the designed molecular and reasonably evaluate the designed molecules using the corresponding pIC_{50} values, converted from the obtained IC_{50} (μM) values of MMP-2 inhibition via built-in QSAR software of DS 3.5 (Discovery Studio 3.5, Accelrys, Co. Ltd). The training and test set was divided in diverse molecules module of DS 3.5, in which the test set includes four compounds (**E5, E8, E10, E15**) while other 14 compounds were classified into the training set. The 3D-QSAR model defined the critical regions (steric or electrostatic) affecting the binding affinity. It was a PLS model set up 400 independent variables (conventional $R^2 = 0.92$) which proved the reliability of this model.

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Table 3 Data based on molecular simulation of 3D-QSAR of compounds **E1-E18**

Compound [a]	MMP-2		Residual error
	Actual pIC_{50}	Predicted pIC_{50}	
E1	4.67	4.68	-0.0061
E2	4.46	4.46	0.0042
E3	4.55	4.55	0.00013
E4	4.95	4.95	0.00038
E5	5.08	4.795	0.289
E6	4.32	4.325	-0.0046
E7	5.02	5.02	0.0012
E8	4.91	4.72	0.19
E9	4.79	4.79	-0.0032
E10	4.97	4.73	0.24
E11	5.17	5.16	0.0051
E12	4.23	4.23	0.0018
E13	4.89	4.89	0.0014
E14	4.77	4.77	-0.0015
E15	4.86	4.87	-0.013
E16	4.93	4.93	0.0013
E17	5.55	5.55	-0.0024
E18	4.24	4.24	0.0022

[a] Underlined for the test set, the rest belonging to the training set.Practical test and the 3D-QSAR model predicted $pIC_{50\ MMP\text{-}2}$ values of compounds **E1-E18**, and the residual error values were summarized in **Table 3.** Based on the linear relationship between them in Fig. 3a, this model had a good forecasting ability. A contour plot of the electrostatic field region, enhancing anticancer activity, was in blue which means that the electron density increases, but red converse Fig. 3b. As shown in Fig. 3c, the energy grids corresponding to the favorable (in green) or unfavorable (yellow) steric effects for the MMP-2 affinity. As illustrated in these pictures, this QSAR model predicted structure-activity relationship of the ring substituents on R_2 was consistent with the actual value. It also offers the possibility to optimize the molecular structure of the inhibitor, like introducing large volume of electron-donating substituents on the benzene ring to which R1 is attached and small volume of electron-donating substituents of R_2. This promising model would give directions to design and optimize more potent MMP-2 inhibitors based on the dihydropyrazothiazole bearing salicylaldehyde groups and contributed to our further study in future.

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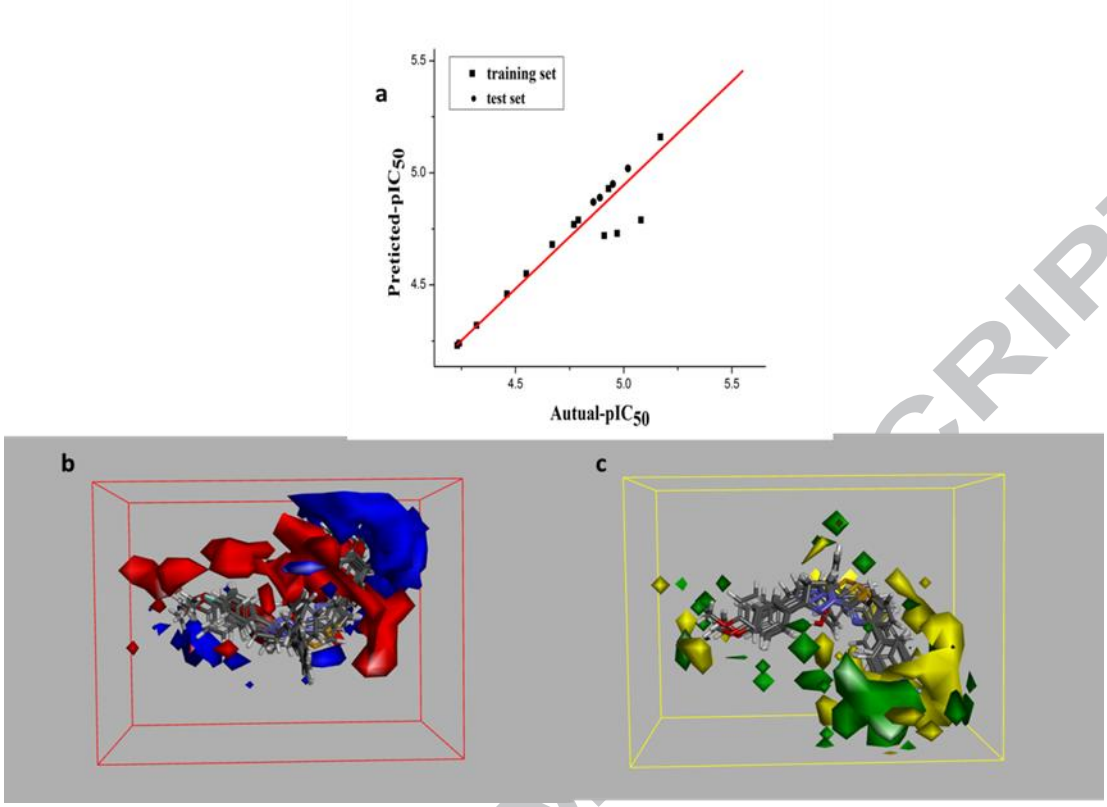

Fig. 3. (a) The pIC50 regression curve based on the predictive and experimental value of MMP-2 inhibitor (b) Isosurface of the 3D-QSAR model coefficients on electrostatic potential grids. (c) Isosurface of the 3D-QSAR model coefficients on Van der Waals grids.

In this study, a series of salicylaldehyde containing dihydropyrazole thiazole derivatives **E1-E18** have been designed, synthesized and evaluated for their anti-proliferative activities against MCF-7, HeLa and HepG2 cells. Most of them exhibited more anti-proliferative activities than positive control drugs **Gefitinib** and **Celecoxib**. We also evaluated these target compounds for their selective MMP-2 inhibitory activities. Among them, compound **E17** showed the most potent MMP-2 inhibition activities (IC_{50} = 2.80 μM). The probable binding models were obtained by docking simulation, suggesting that the dihydropyrazole thiazole and salicylaldehyde backbone are favorable for the zinc-chelating bonds and π-cation bond which provide considerable steric binding stabilization. But beyond that, 3D-QASR models were built with previous activity data and binding conformations to begin our work in this paper as well as to provide a reliable tool for reasonable design and synthesis of potent MMP-2 inhibitors. Furthermore, we will continue to optimize these compounds in the hope of obtaining drugs with highly effective.

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Acknowledgments

This work was supported by the National Key Research and Development Program of China (2016YFD0200207).

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Dihydropyrazothiazole derivatives as potential MMP-2/MMP-8 inhibitors for cancer therapy

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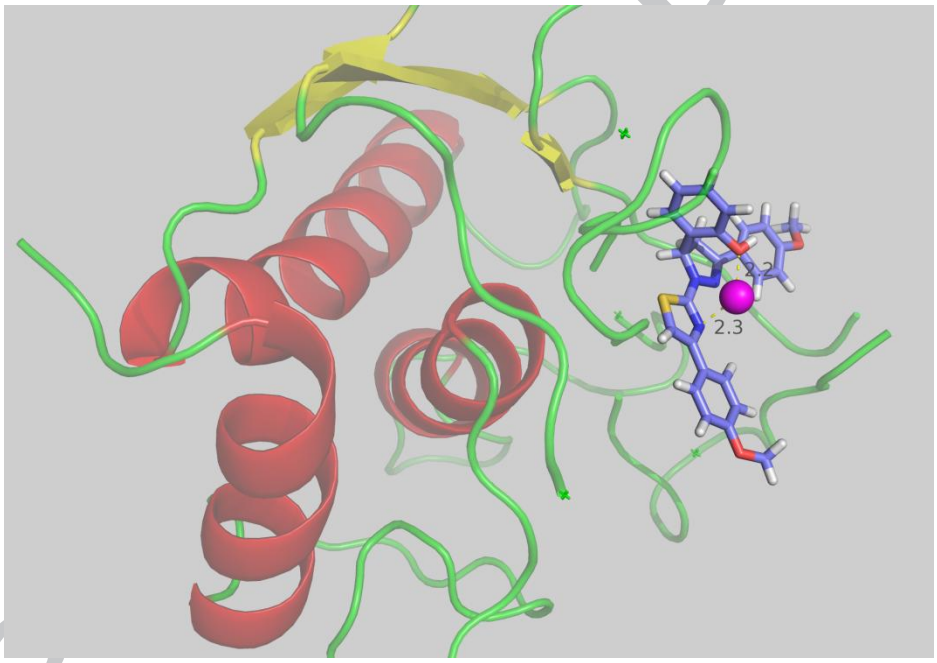

Interactions between compound **E16** and MMP-2 in a three dimentional stereogram

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> 18 dihydropyrazothiazole derivatives had been synthesized.

> Most of the compounds showed low toxicity to 293T/LO2 cells.

> Compound **E17** showed the most potent and best selective MMP-2 inhibition.

> We built a 3D-QASR model for the further explanation of SAR about these compounds.

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