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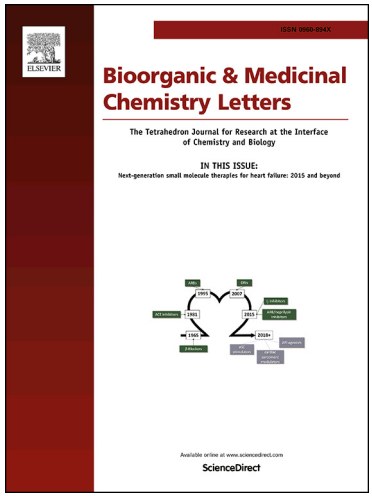

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Synthesis and biological evaluation of acylthiourea against DUSP1 inhibition

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Key Words: Dual specificity phosphatase, Mitogen-activated protein kinase, Enzyme inhibition, Structure activity relationship, Cancer

Abstract: Structure based virtual screening attempts to discover DUSP1 inhibitors have yielded a scaffold featuring benzoxazole and acylthiourea pharmacophore. A series of its analogues were synthesized to explore structure activity relationship (SAR) of DUSP1 inhibition.

Dual specificity phosphatases (DUSPs) represent a large family of enzymes that are able to dephosphorylate both phospho-tyrosine and phospho-threonine residues on mitogen-activated

protein kinases (MAPKs).[1,2] Because the MAPK family play a critical role in the cellular signalling pathways leading to the regulation of cell growth and inflammation,[3] regulating MAPK-mediated pathways is a therapeutically attractive strategy. DUSP1, known as mitogen-activated protein kinase phosphatase 1 (MKP1), is frequently activated in a variety of human diseases including cancers,[4] depression,[5] arthritis,[6] Alzheimer's disease[7] and asthma.[8] In this regard, DUSP1 represents attractive potential drug targets.

Three-dimensional structure of DUSP1 was reported in complex with a substrate analogue (PDB entry: 6APX).[9] The presence of structural information on the interaction of a small-molecule ligand in the active site would make it possible to design the competitive inhibitors that can develop into a drug candidate. The discovery of druggable inhibitors can be accelerated to a great extent when assisted with the effective computer-aided molecular design.

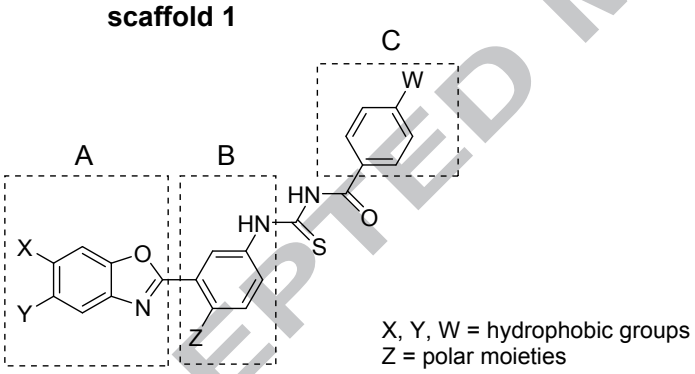
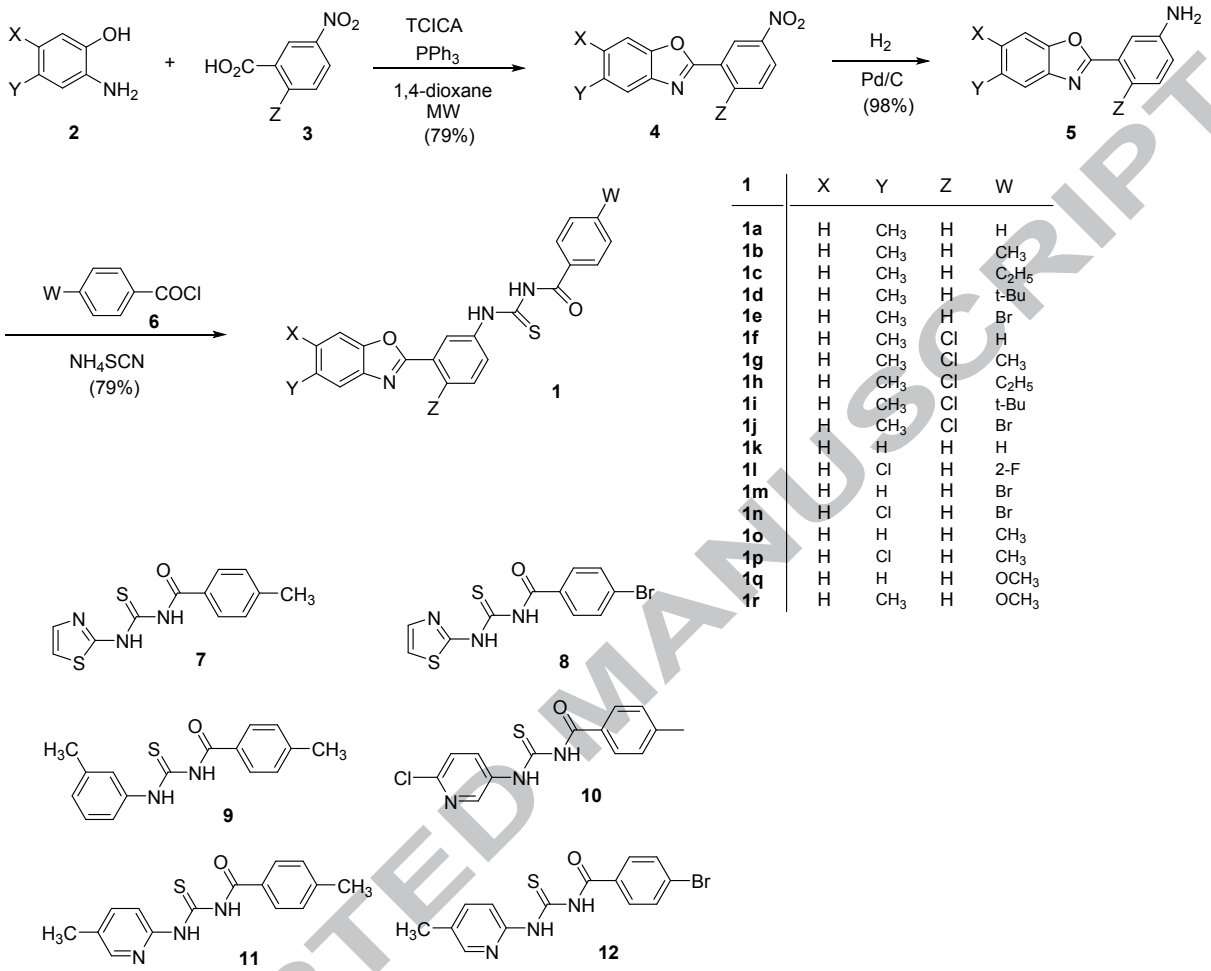


Figure 1. Structure of scaffold 1 (**1**).

Structure based virtual screening with the automated AutoDock program[10] yielded a few weak DUSP1 inhibitors sharing the molecular core shown in Figure 1. The structure of scaffold 1 (**1**) features benzoxazole and acylthiourea pharmacophore. Herein, we designed and synthesized its analogues to explore structure activity relationship (SAR) of DUSP1 inhibition for a potential small molecule lead in the development of new therapeutics against cancers and neurological diseases.



1	X	Y	Z	W
1a	H	CH_3	H	H
1b	H	CH_3	H	CH_3
1c	H	CH_3	H	C_2H_5
1d	H	CH_3	H	t-Bu
1e	H	CH_3	H	Br
1f	H	CH_3	Cl	H
1g	H	CH_3	Cl	CH_3
1h	H	CH_3	Cl	C_2H_5
1i	H	CH_3	Cl	t-Bu
1j	H	CH_3	Cl	Br
1k	H	H	H	H
1l	H	Cl	H	2-F
1m	H	H	H	Br
1n	H	Cl	H	Br
1o	H	H	H	CH_3
1p	H	Cl	H	CH_3
1q	H	H	H	OCH_3
1r	H	CH_3	H	OCH_3

Scheme 1. Synthesis of scaffold 1 analogues.

Our synthetic approach to the scaffold 1 was outlined in Scheme 1. Commercially available 2-aminophenol **2** was reacted with 3-nitrobenzoic acid **3** in the presence of trichloroisocyanuric acid (TCICA) and PPh_3 in 1,4-dioxane under microwave irradiation to yield benzoxazole **4**. When the reaction mixture was refluxed under classical heating, the intermediate amide prior to the formation of benzoxazole was mainly formed even at longer reaction times. However, heating by microwave irradiation enhanced the benzoxazole formation in terms of yield and short reaction times. Reduction of the nitro group in compound **4** with Pd/C afforded amine **5**, which was reacted in refluxing acetone with the

intermediate aroyl thiocyanate formed by treatment of acyl chloride **6** with ammonium thiocyanate, to afford acylthiourea **1** in 60-95% yield.

Table 1. Inhibitory activity of synthesized compounds against DUSP1

Compd	MKP1(20μM)	VHR(20μM)	MKP1(IC_{50})
1a	23	34	16
1b	26	16	6.6
1c	47	50	21
1d	91	80	-
1e	10	20	7.9
1f	34	44	16
1g	33	19	8.3
1h	52	63	27
1i	88	82	-
1j	14	15	8.7
1k	21	61	25
1l	41	37	17
1m	8	11	4.8
1n	3	5	2.7
1o	42	74	35
1p	52	59	17
1q	53	48	19
1r	19	13	11
7	99	102	-
8	103	103	-
9	98	99	-
10	103	103	-
11	98	101	-
12	95	99	-

To explore structure-activity relationship (SAR) of scaffold 1 (**1)** and DUSP1 inhibition, we synthesized a series of 18 analogues with modifications in three functional groups of the **1** pharmacophore, and additionally 6 analogues lacking benzoxazole moiety. Inhibitory activity of the synthesized compounds against DUSP1 (mitogen-activated protein kinase phosphatase 1 (MKP1)) was listed in table 1. Chemical complementation assays[11] showed that compound **7-12**, which lacked benzoxazole moiety did not inhibit MKP1, although the others **1a-r** inhibited the dual specificity phosphatases MKP1 and VHR. Inhibitory activity of compounds was selective towards either MKP1 or VHR depending on substituents. For example, **1m** and **1n** were specific to MKP1, whereas **1b** and **1r** were specific to VHR, consistent with structural differences in the active site of the two enzymes.[12] The substituents in A moiety (benzoxazole) of scaffold **1** influence the activity, but lacked the correlation between the substituent and biological activity. Electron donating substituent could increase the inhibitory activity (**1a** vs **1k**, **1b vs 1p**) or decrease the activity (**1e** vs **1m** vs **1n**). The substituent in B

moiety did not influence the activity (**1a** vs **1f**, **1b** vs **1g**) and there was no difference (**1a** vs **1f**, **1b** vs **1g**) in inhibitory activity whether the substituent is electron donating or electron withdrawing group. The presence of electron withdrawing group in C moiety increased inhibitory activity against MKP1 (**1e**, **1m**, **1n**). Among the tested compounds, compound **1n** exhibited the strongest inhibitory activity. The SAR studies revealed a strong correlation between the substituent of C moiety and inhibition of cellular DUSP1. The existence of electron withdrawing substituent in C moiety of scaffold **1** was crucial to inhibitory activity against mitogen-activated protein kinase phosphatase 1 (MKP1).

In conclusion, a series of novel acylthiourea derivatives were synthesized through efficient synthetic routes and evaluated for inhibitory activity against mitogen-activated protein kinase phosphatase 1 (MKP1). The SAR studies revealed that the existence of electron withdrawing substituent in C moiety of scaffold **1** was crucial to the inhibitory activity. Among all the compounds designed, compound **1n** possessing a bromine functional group showed the strongest inhibitory activity.

Acknowledgements

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11. The gene for catalytic domain of DUSP1 corresponding to residues 152-323 was cloned into NdeI and XhoI restriction sites of the expression vector pET21b. Cells containing the vector were induced with 0.1mM IPTG and further grown at 18 °C for 16 hours. Cell pellets were resuspended in the lysis buffer containing 50 mM Tris-HCl (pH 7.5), 0.5 M NaCl, 1mM PMSF, and 0.05% β-mercaptoethanol. The resuspension was then lysed by sonication on ice. The His-tagged DUSP1 catalytic domain was purified by a TALON crude column and dialyzed against 20 mM Tris-HCl (pH 8.0), 0.5 mM EDTA, 200 mM NaCl and 10 mM β-mercaptoethanol. The enzyme assay was performed by monitoring the extent of hydrolysis of 6,8-difluoro-4-methyl-umbelliferyl phosphate (DiFMUP) with spectrofluorometric analysis. The purified DUSP1 (20 nM), DiFMUP (2.5 μM), and candidate inhibitors were incubated in the reaction mixture containing 20 mM Tris-HCl (pH 8.0), 0.01% Trition X-100, and 5 mM DTT for 30 minutes. The resulting fluorescence was measured by using the Perkin Elmer 2030 multiplate reader with excitation and emission wavelengths of 355 nm and 460 nm, respectively. IC_{50} values of the potent inhibitors were determined from direct regression curve analysis in duplicate.

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Graphical Abstract

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