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Research Letter

Synthesis and Biological Evaluation of 7-O-Modified Formononetin Derivatives

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Three series of novel formononetin derivatives were synthesized, in which formononetin and heterocyclic moieties were separated by 2-carbon, 3-carbon, and 4-carbon spacers. The chemical structures of these compounds were confirmed. All the derivatives were screened for antiproliferative activities against Jurkat cell line and HepG-2 cell line. In this paper, compounds prepared were also screened for their antibacterial activity of six bacterial strains. Compound **3b** exihibited promising antibacterial activity against *B. subtilis* with minimal inhibitory concentration (MIC) value of 0.78 μg/mL, and compound **5e** showed significant antiproliferative activities against Jurkat cell growth with IC_{50} of 1.35×10^{-4} μg/mL. The preliminary investigation of structure-activity relationships (SARs) was also discussed based on the obtained experimental data.

1. Introduction

Isoflavonoids are a broad class of polyphenolic secondary metabolites that are abundant in plants [1, 2] and in various common foods such as apples, onions, tea, and red wine [3, 4]. Isoflavonoids also have a potent activity against protein tyrosine kinase (PTK) [5]. Because of such a broad range of pharmacological properties, they receive considerable therapeutic importance. Protein tyrosine kinases (PTKs) have been intensively investigated because of their role in the transduction of proliferative signals in mammalian cells. Many transmembrane growth factor receptors possess intracellular PTK activity, with initiation of this activity following external binding of a growth factor, being the first step in the cellular signal transduction pathway which controls mitogenesis and cell proliferation [6, 7]. Therefore, selective interruption of signal transduction in tumor cells by specific inhibitors of PTK activity has recently emerged as a major new approach for the design of tumor-specific drugs [8, 9].

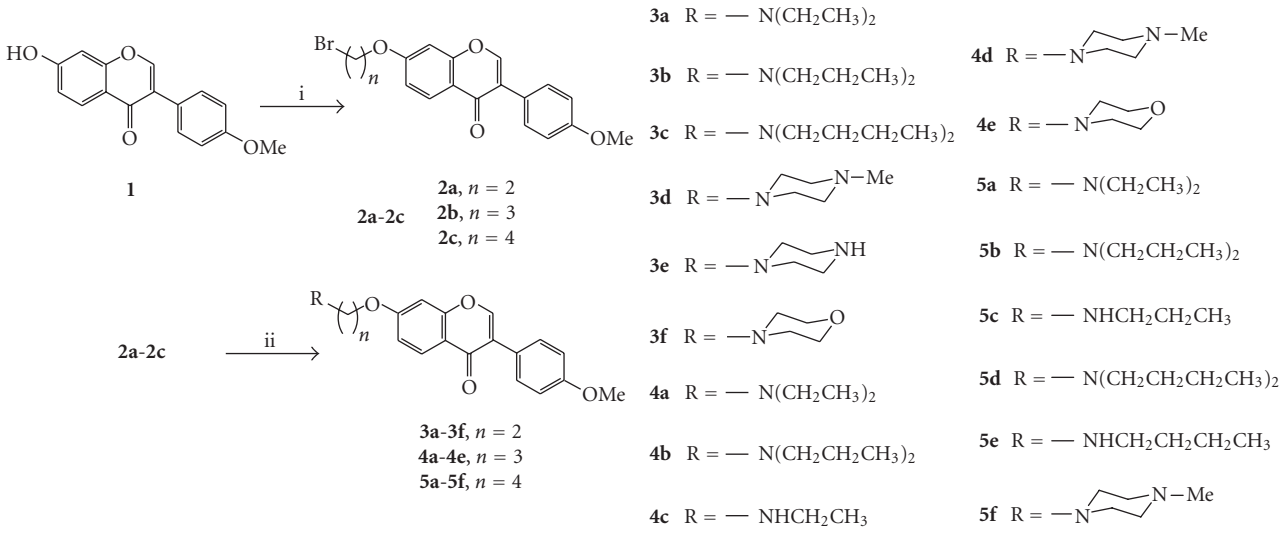
Formononetin (**1**, shown in Scheme 1), a kind of isoflavonoid, is reported to have many biological activities including antiproliferative, antioxidant, antidiabetic, antiestrogenic, antibacterial, antiangiogenic effects, and so on [10–14]. Formononetin is also a potent aryl hydrocarbon receptor agonist in vitro [14]. The versatile biological activities of formononetin prompt us to prepare a new series of its derivatives and evaluate their biological significance. Herein, we describe the synthesis of formononetin derivatives in which formononetin and heterocyclic moieties were linked by spacers,and investigate the effects of the size of the spacers and substitution patterns of the heterocyclic moieties. All of the compounds were assayed for their antiproliferative activities against a panel of two human tumor cell lines (Jurkat and HepG-2) by applying the MTT colorimetric assay. The results of this study may be useful to researchers attempting to gain more understanding of the PTK inhibitory activity of isoflavonoid derivatives.

To our knowledge, this is the first report on the screening of 7-O-modified formononetin derivatives for their antimicrobial and antiproliferative activities.

2. Results and Discussions

2.1. Chemical Synthesis

Compounds **2a–c** were the key intermediates for the synthesis of the compounds investigated. They were prepared



Scheme 1: Synthesis of 7-O-heterocycle derivatives of formononetin. Reagents and conditions: (i) $BrCH_2CH_2Br$, $BrCH_2CH_2CH_2Br$ or $BrCH_2CH_2CH_2CH_2Br$, K_2CO_3, DMF; (ii) R, K_2CO_3, dioxane, DMF, heating.

from alkylation of 7-OH group by using 1,2-, 1,3-, or 1,4-dihaloalkanes in the presence of K_2CO_3 in anhydrous DMF [15]. The synthesis of compounds **3a–f**, **4a–e**, and **5a–f** was accomplished according to the general pathway illustrated in Scheme 1. To increase the antimicrobial properties of formononetin, formononetin derivatives in which the formononetin ring system was linked to the alkylamines by different spacers at C-7 position were investigated, with a view to modify their lipophilicity. Literature survey revealed that the compounds containing alkyl amino side chains showed better activities against the test bacteria than those containing aromatic ring amino side chains [15]. To further optimize this activity, seventeen compounds reported in this paper contain alkyl amino groups. Reaction of **2a–c** with different cyclic and noncyclic alkylamines yielded **3a–f**, **4a–e**, and **5a–f**, respectively, which were all first reported. All of the synthesized compounds gave satisfactory analytical and spectroscopic data, which were in full accordance with their depicted structures.

2.2. Biological Evaluation and Discussion

2.2.1. Antiproliferative Activities [16]

The antiproliferative activities of these compounds were evaluated against a panel of two human tumor cell lines (Jurkat and HepG-2) by applying the MTT colorimetric assay. The observed IC50's are listed in Table 1. From the results of the in vitro antiproliferative MTT tests of the prepared compounds, it followed that in series 1, most of the prepared compounds showed good antiproliferative activities. Among them compounds **3b** and **3d** exhibited strong activities on Jurkat cell growth. Also, compounds **3b** and **3c** had stronger activities on HepG-2 cell line than the positive control 5-UF. In series 2, compounds **4a**, **4b**, and **4e** displayed remarkable antiproliferative activities on Jurkat cell, while they just showed moderate activities against HepG-2 cell. In series 3, compound **5e** displayed significant antiproliferative activities on Jurkat cell growth,

Table 1: Antiproliferative activity of the synthesized compounds.

Compounds	IC_{50} (μg/mL)	
	Jurkat[1]	HepG-2[2]
1	23.52	73.03
2a	48.04	>100.00
2b	0.21	4.14
2c	9.50	7.18
3a	13.93	5.57
3b	1.82	3.24
3c	>100.00	2.67
3d	3.70	8.14
3e	12.21	7.15
3f	16.49	7.54
4a	0.41	6.14
4b	5.32	8.58
4c	16.44	52.21
4d	18.08	5.90
4e	3.49	57.82
5a	1.94	6.54
5b	>100.00	7.52
5c	0.64	4.94
5d	1.27	1.49
5e	1.35×10^{-4}	13.14
5f	7.11	13.53
5-fluorouracil	18.41	2.50

[1]Jurkat: Human T cell lymphoblast-like cell line.
[2]HepG-2: Human hepatocellular liver carcinoma cell line.

Table 2: Antimicrobial activity of the synthesized compounds.

Compounds	Minimum inhibitory concentrations MICs (μg/mL)					
	Gram positive			Gram negative		
	S. faecalis	*S. aureus*	*B. subtilis*	*E. coli*	*P. aeruginosa*	*E. cloacae*
1	3.12	25.00	50.00	50.00	25.00	50.00
2a	3.12	6.25	3.12	6.25	1.56	50.00
2b	3.12	50.00	25.00	6.25	25.00	1.56
2c	3.12	3.12	50.00	12.50	25.00	12.50
3a	3.12	6.25	50.00	3.12	3.12	25.00
3b	1.56	12.50	0.78	3.12	50.00	3.12
3c	3.12	6.25	6.25	25.00	6.25	3.12
3d	3.12	6.25	6.25	12.50	3.12	6.25
3e	3.12	1.56	50.00	25.00	25.00	6.25
3f	3.12	12.50	25.00	6.25	50.00	25.00
4a	3.12	3.12	50.00	6.25	50.00	50.00
4b	3.12	25.00	3.12	1.56	6.25	12.50
4c	3.12	12.50	25.00	6.25	6.25	12.50
4d	3.12	6.25	3.12	12.50	3.12	25.00
4e	3.12	3.12	50.00	1.56	50.00	50.00
5a	3.12	1.56	6.25	25.00	6.25	3.12
5b	3.12	12.50	1.56	25.00	50.00	50.00
5c	3.12	50.00	1.56	6.25	6.25	50.00
5d	3.12	50.00	25.00	6.25	3.12	6.25
5e	3.12	0.78	12.50	6.25	25.00	25.00
5f	3.12	25.00	50.00	50.00	3.12	1.56
Penicillin	1.56	1.56	1.56	6.25	6.25	3.12
Kanamycin	3.12	1.56	0.39	3.12	3.12	1.56

and compound **5d** showed promising inhibitory activities on HepG-2 cell growth.

2.2.2. Antibacterial Activities [17]

The antibacterial activities of the synthetic compounds were tested against *B. subtilis*, *S. aureus* and *S. faecalis* (Gram-positive bacteria), *E. coli*, *P. aeruginosa,* and *E. cloacae* (Gram-negative bacteria) by broth dilution method recommended by National Committee for Clinical Laboratory Standards (NCCLS) [18, 19]. Standard antimicrobial agents like penicillin and kanamycin were also screened under identical conditions for comparison. The minimal inhibitory concentration (MIC) values for the bacteria are listed in Table 2.

Most compounds in series 1, which contained a 2-carbon spacer, displayed good activities against the test microorganisms. In this series, compound **3b** showed pronounced activity against *B. subtilis* and *S. faecalis* with MIC values of 1.56 μg/mL and 0.78 μg/mL, respectively. In addition, compound **3e** showed good activity against *S. aureus*.

A few compounds in series 2, which contain a 3-carbon spacer, exhibited great activities against the test microorganisms. In this series, compounds **4b**, **4d**, and **4e** showed great activities against *S. aureus*, *B. subtilis,* and *E. coli*. Among them, **4b** and **4e** showed strong activities against *E. coli* with their MIC value (1.56 μg/mL) superior to the positive control kanamycin.

Similarly, several compounds in series 3, which contain a 4-carbon spacer, showed good activities against the test microorganisms. Among them, compounds **5a** and **5e** exhibited strong activities against *S. aureus* with their MIC values of 1.56 μg/mL and 0.78 μg/mL, respectively. Compounds **5b** and **5c** displayed great activities against *B. subtilis*. Also, compounds **5d** and **5f** showed strong activities against *P. aeruginosa* with the MICs (3.12 μg/mL), which were comparable to the positive control kanamycin.

3. Conclusion

To investigate the biological activities of formononetin derivatives, we had synthesized three series of formononetin derivatives. For all the compounds synthesized, antiproliferative and antibacterial activities against two cancer cell lines (Jurkat and HepG-2) and six bacterial strains (three Gram-positive bacterial strains: *Bacillus subtilis*, *Staphylococcus aureus*, and *Streptococcus faecalis* and three Gram-negative bacterial strains: *Escherichia coli*, *Pseudomonas aeruginosa*, and *Enterobacter cloacae*) were determined. From the bioassay results, it may be concluded that those containing long

alkyl amino side chains exhibited better activities against gram-positive bacteria than those containing short ones, while it had the opposite rule for gram-negative. Specifically, the derivatives with dipropylamine moiety were more active than most of the other analog. In this study, we focused our attention on the structure-activity relationships. The work was of interest because this is a preliminary investigation of SAR, serving as the basis of further more detailed work.

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References

[1] V. M. Malikov and M. P. Yuldashev, "Phenolic compounds of plants of the *Scutellaria* genus. Distribution, structure, and properties," *Chemistry of Natural Compounds*, vol. 38, no. 5, pp. 473–519, 2002.

[2] M. J. del Baño, J. Lorente, J. Castillo, et al., "Flavonoid distribution during the development of leaves, flowers, stems, and roots of *Rosmarinus officinalis*. Postulation of a biosynthetic pathway," *Journal of Agricultural and Food Chemistry*, vol. 52, no. 16, pp. 4987–4992, 2004.

[3] P. L. Whitten, S. Kudo, and K. K. Okubo, "Isoflavonoids," in *Handbook of Plant and Fungal Toxicants*, pp. 117–137, CRC Press, Boca Raton, Fla, USA, 1997.

[4] G. M. Boland and D. M. X. Donnelly, "Isoflavonoids and related compounds," *Natural Product Reports*, vol. 15, no. 3, pp. 241–260, 1998.

[5] K. T. Papazisis, D. Zambouli, O. T. Kimoundri, et al., "Protein tyrosine kinase inhibitor, genistein, enhances apoptosis and cell cycle arrest in K562 cells treated with γ-irradiation," *Cancer Letters*, vol. 160, no. 1, pp. 107–113, 2000.

[6] A. Ullrich and J. Schlessinger, "Signal transduction by receptors with tyrosine kinase activity," *Cell*, vol. 61, no. 2, pp. 203–212, 1990.

[7] S. R. Hubbard and J. H. Till, "Protein tyrosine kinase structure and function," *Annual Review of Biochemistry*, vol. 69, pp. 373–398, 2000.

[8] F. M. Uckun and C. Mao, "Tyrosine kinases as new molecular targets in treatment of inflammatory disorders and leukemia," *Current Pharmaceutical Design*, vol. 10, no. 10, pp. 1083–1091, 2004.

[9] P. Traxler, J. Green, H. Mett, U. Séquin, and P. Furet, "Use of a pharmacophore model for the design of EGFR tyrosine kinase inhibitors: isoflavones and 3-phenyl-4(1*H*)-quinolones," *Journal of Medicinal Chemistry*, vol. 42, no. 6, pp. 1018–1026, 1999.

[10] X. Yu, W. Wang, and M. Yang, "Antioxidant activities of compounds isolated from *Dalbergia odorifera T. Chen* and their inhibition effects on the decrease of glutathione level of rat lens induced by UV irradiation," *Food Chemistry*, vol. 104, no. 2, pp. 715–720, 2007.

[11] Y. Ungar, O. F. Osundahunsi, and E. Shimoni, "Thermal stability of genistein and daidzein and its effect on their antioxidant activity," *Journal of Agricultural and Food Chemistry*, vol. 51, no. 15, pp. 4394–4399, 2003.

[12] S. Sato, J. Takeo, C. Aoyama, and H. Kawahara, "Na^+-glucose cotransporter (SGLT) inhibitory flavonoids from the roots of *Sophora flavescens*," *Bioorganic & Medicinal Chemistry*, vol. 15, no. 10, pp. 3445–3449, 2007.

[13] Z.-N. Ji, W. Y. Zhao, G. R. Liao, et al., "In vitro estrogenic activity of formononetin by two bioassay systems," *Gynecological Endocrinology*, vol. 22, no. 10, pp. 578–584, 2006.

[14] S. Medjakovic and A. Jungbauer, "Red clover isoflavones biochanin A and formononetin are potent ligands of the human aryl hydrocarbon receptor," *The Journal of Steroid Biochemistry and Molecular Biology*, vol. 108, no. 1-2, pp. 171–177, 2008.

[15] L.-N. Zhang, Z.-P. Xiao, H. Ding, et al., "Synthesis and cytotoxic evaluation of novel 7-*O*-modified genistein derivatives," *Chemistry & Biodiversity*, vol. 4, no. 2, pp. 248–255, 2007.

[16] J. Meletiadis, J. F. G. M. Meis, J. W. Mouton, J. P. Donnelly, and P. E. Verweij, "Comparison of NCCLS and 3-(4,5-dimethyl-2-thiazyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) methods of in vitro susceptibility testing of filamentous fungi and development of a new simplified method," *Journal of Clinical Microbiology*, vol. 38, no. 8, pp. 2949–2954, 2000.

[17] M. Hajdúch, V. Mihál, J. Minařík, et al., "Decreased in vitro chemosensitivity of tumour cells in patients suffering from malignant diseases with a poor prognosis," *Cytotechnology*, vol. 19, no. 3, pp. 243–245, 1996.

[18] National committee for clinical laboratory standards, "Reference methed for broth dilution antifungal susceptibility testing of yeasts: approved standard," M27-A, NCCLS, Wayne, Pa, USA, 1997.

[19] National committee for clinical laboratory standards, "Development of in vitro susceptibility testing criteria and quality control parameters: tentative guideline," M23-T3, NCCLS, Villanova, Pa, USA, 1998.