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SHORT COMMUNICATION

Rho-kinase inhibitors from adlay seeds

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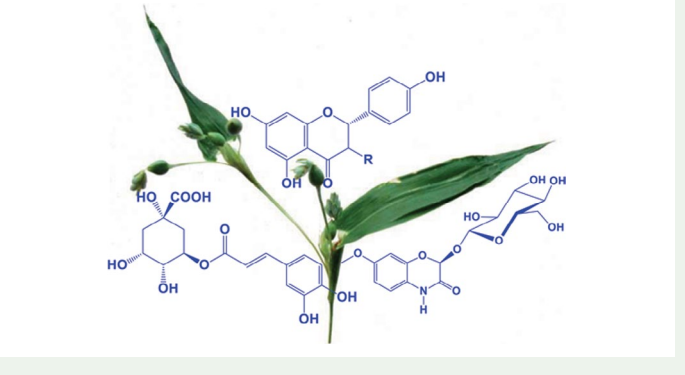
ABSTRACT

Rho-kinase enzymes are one of the most important targets recently identified in our bodies. Several lines of evidence indicate that these enzymes are involved in many diseases and cellular disorders. ROCK inhibitors may have clinical applications for cancer, hypertension, glaucoma, etc. Our study aims to identify the possible involvement of Rho-kinase inhibition to the multiple biological activities of adlay seeds and provide a rationale for their folkloric medicines. Hence, we evaluated Rho-kinase I and II inhibitory activity of the ethanol extract and 28 compounds derived from the seeds. A molecular docking assay was designed to estimate the binding affinity of the tested compounds with the target enzymes. The results of our study suggest a possible involvement of Rho-kinase inhibition to the multiple biological activities of the seeds. Furthermore, the results obtained with the tested compounds revealed some interesting skeletons as a scaffold for design and development of natural Rho-kinase inhibitors.



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1. Introduction

Adlay (Job's tears or Hato Mugi; *Coix lacryma-jobi,* Poaceae) has been consumed in traditional Chinese medicine to treat inflammation, dysfunction of the endocrine system, topically for the treatment of warts, chapped skin, rheumatism and neuralgia. They also serve as diuretic, antihyperuricemia, antitumour, antimelanogenic and analgesic agent besides being a nutritive food supplement in many Asian countries (Seo et al. 2000; Chung et al. 2011; Li et al. 2014; Zhao et al. 2014; Amen, Arung et al. 2017). The multiple effects of adlay seeds against these diseases are attributed to the bioactive constituents having wide pharmacological activities. Rho-kinase enzymes are recently discovered as one of the most important targets in our bodies. Over-expression of these enzymes is involved in the pathogenesis of a wide range of diseases and ROCK inhibitors may have clinical applications for cancer, obesity, hypertension, diabetes, glaucoma, erectile dysfunction, etc. (Pan et al. 2013). Recently, many synthetic Rho-kinase inhibitors were developed as therapeutic targets for many diseases, while the supply of these inhibitors from natural products is still on the way. Taking into consideration the involvement of Rho-kinase inhibition in many diseases and cellular disorders, we suggest that the potential activities of the extract of *C. lacryma-jobi* seeds are mediated in part through Rho-kinase inhibition. In the course of our ongoing efforts to discover natural Rho-kinase inhibitors (Amen, Zhu et al. 2017), we utilised an *in vitro* bioassay to investigate whether the inhibition of Rho-kinases contribute to the wide range of versatile activities of adlay seeds or not. In addition, which of the compounds are responsible for the inhibition of Rho-kinases?

2. Results and discussion

The activity of the total ethanol extract of *C. lacryma-jobi* seeds at a concentration of 1 mg/mL, was tested against the two enzymes (ROCK-1 and ROCK-II). The inhibition percentages against ROCK-I and ROCK-II were 55.2 ± 2.6 (IC_{50} 1001.53 ± 4.7 µg/mL) and 53.37 ± 4.1 (IC_{50} 1000.91 ± 5.3 µg/mL), respectively. Twenty-eight compounds (Figure 1) derived from the seeds, were tested against the target enzymes to find the potential compounds responsible for the inhibitory activity of the extract. The ROCK inhibitory activity and the docking scores of the tested compounds are presented in Table 1. Y-27632 was used as a standard non-specific inhibitor of ROCK-I and ROCK-II. It showed inhibitory percentages of 44.7 ± 2.6 and 41.8 ± 4.6, respectively at a final concentration of 1.6 µM. Notably, β-sitosterol **(5)**, stigmasterol **(6)**, chlorogenic acid **(15)**, 2-*O*-β-glucopyranosyl-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one **(19)** and the tested flavonoids **(23–27)** exhibited a moderate to strong inhibition of the activity of the two enzymes at a final concentration of 100 µM. β-sitosterol **(5)** and stigmasterol **(6)**, the common plant sterols, showed a selective ROCK-I inhibitory activity with inhibitory percentages of 35.8 ± 1.5 and 40.9 ± 2.8, respectively. Chlorogenic acid **(15)** and the tested flavonoids **(23–27)** showed variable activities against Rho-kinase enzymes with an inhibition percentage about 40%. The rest of the compounds showed weak inhibition of the activity of the enzymes. A molecular docking experiment was designed to investigate the binding of the compounds to the target enzymes. Interestingly, the data revealed a parallel correlation between the ROCK inhibitory activity of most of the tested compounds and their binding affinity to the target enzymes (Table 1). It is worth to note that 2-O-β-glucopyranosyl-7-methoxy-2H-1,4-benzoxazin-3(4H)-one **(19)** represents itself as the most active compound

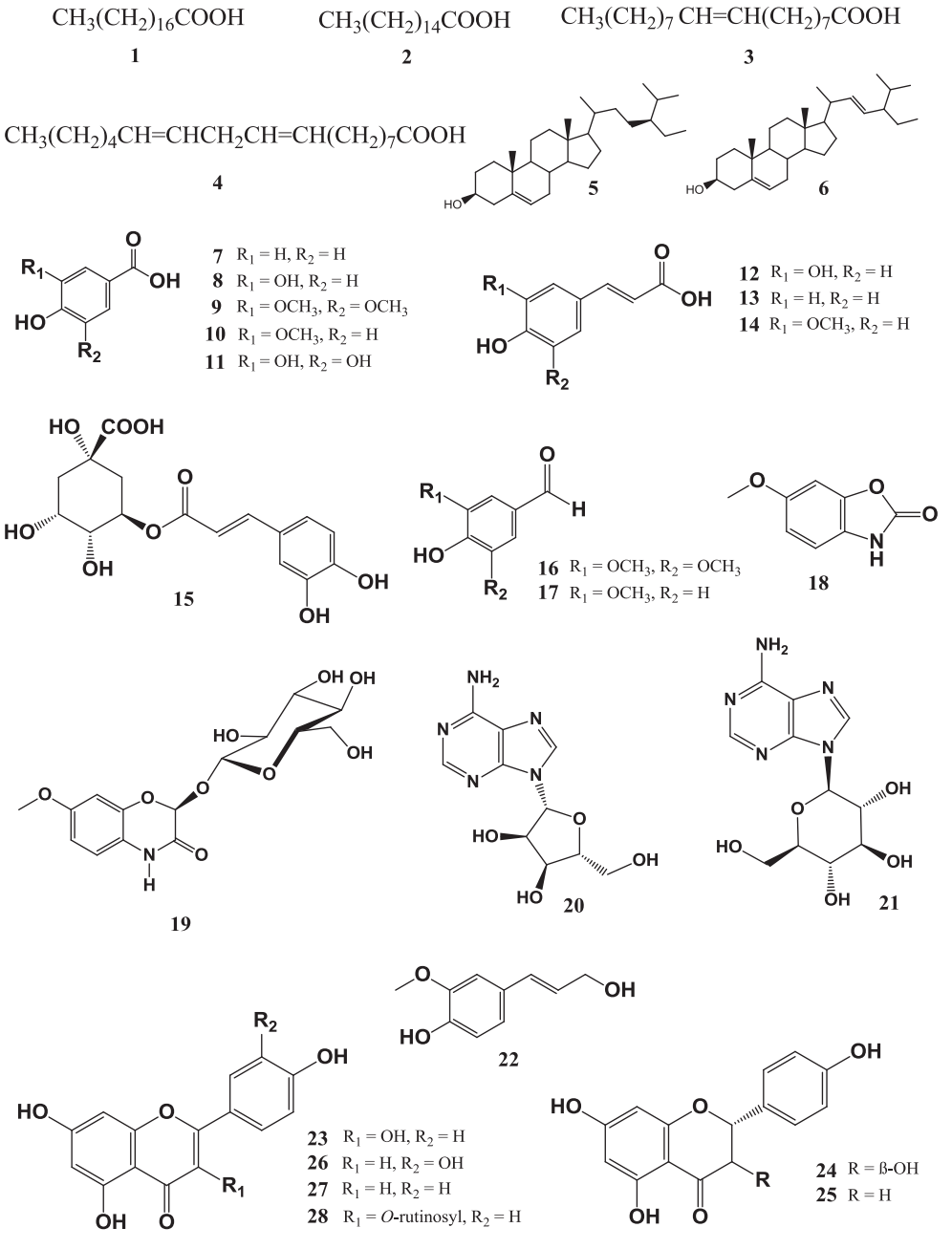

Figure 1. The structures of compounds **(1–28)** from the seeds of *C. lacryma-jobi*.

with inhibition percentages of 53.9 ± 2.5 (IC_{50} 98.75 ± 3.9 μM) and 54.8 ± 4.8 (IC_{50} 98.86 ± 2.7 μM) against ROCK-I and ROCK-II, respectively as well as showing a strong binding affinity to the target enzymes (Table 1, Figure S1 and Figure S2). The compound has a unique structure and should encourage further studies for the development of natural Rho-kinase inhibitors.

Table 1. ROCK inhibitory activity and docking scores of the tested compounds from *C. lacryma-jobi* seeds.

		ROCK assay*		Docking experiment**	
	Compounds	ROCK-I Inhibition (%)	ROCK-II Inhibition (%)	2ETR (ROCK-I)	2H9 V (ROCK-II)
1	Stearic acid	20.5 ± 2.9	19.9 ± 1.8	−60.11	−41.75
2	Palmitic acid	22.5 ± 1.4	20.8 ± 3.6	−58.02	−41.94
3	Oleic acid	18.6 ± 2.9	15.9 ± 4.9	−61.98	−45.60
4	Linoleic acid	24.7 ± 2.2	26.7 ± 3.9	−63.42	−48.66
5	**β-sitosterol**	**35.8 ± 1.5**	**Nil*** **	**−70.78**	**−37.15**
6	**Stigmasterol**	**40.9 ± 2.8**	**Nil*** **	**−70.91**	**−38.21**
7	*p*-hydroxy benzoic acid	13.1 ± 4.6	12.9 ± 2.3	−34.77	−30.51
8	Protocatechuic acid	9.1 ± 1.9	10.8 ± 2.7	−36.46	−32.50
9	Syringic acid	10.4 ± 2.8	9.3 ± 1.9	−36.01	−28.62
10	Vanillic acid	12.5 ± 1.6	11.9 ± 3.9	−36.87	−30.17
11	Gallic acid	14.9 ± 3.8	16.7 ± 2.5	−36.81	−33.19
12	Caffeic acid	23.5 ± 4.3	29.9 ± 2.8	−45.89	−35.46
13	*Trans p*-coumaric acid	23.7 ± 1.7	20.8 ± 5.4	−41.36	−32.41
14	*Trans*-ferulic acid	25.4 ± 2.9	22.6 ± 1.7	−43.07	−28.61
15	**Chlorogenic acid**	**36.8 ± 2.9**	**34.8 ± 4.7**	**−53.09**	**−40.22**
16	Syringaldehyde	9.1 ± 4.6	8.6 ± 2.5	−33.44	−26.79
17	Vanillin	11.6 ± 2.9	8.5 ± 3.9	−36.94	−27.79
18	Coixol	9.8 ± 1.4	10.4 ± 2.8	−34.16	−25.13
19	**2-*O*-β-glucopyranosyl-7-methoxy-2*H*-1,4-benzoxaz-in-3(4*H*)-one**	**53.9 ± 2.5 (IC_{50} 98.75 ± 3.9 μM)**	**54.8 ± 4.8 (IC_{50} 98.86 ± 2.7 μM)**	**−54.11**	**−43.58**
20	Adenosine	23.7 ± 3.8	22.9 ± 2.3	−42.48	−32.11
21	9-β-D-Glucopyranosyl adenine	30.2 ± 3.9	17.9 ± 1.3	−43.01	−37.09
22	Coniferyl alcohol	19.4 ± 2.7	20.7 ± 5.1	−42.65	−29.89
23	**Kaempferol**	**33.1 ± 2.5**	**31.6 ± 1.9**	**−48.02**	**−37.03**
24	**(+)-Catechin**	**35.4 ± 1.6**	**37.9 ± 2.7**	**−46.42**	**−28.44**
25	**Naringenin**	**40.4 ± 3.4**	**41.5 ± 2.9**	**−50.22**	**−36.61**
26	**Luteolin**	**39.5 ± 2.8**	**34.1 ± 4.6**	**−49.02**	**−35.77**
27	**Apigenin**	**32.5 ± 2.9**	**31.8 ± 4.4**	**−48.67**	**−35.50**
28	**Rutin**	**41.7 ± 4.6**	**39.8 ± 3.1**	**−59.02**	**−49.61**
	Positive control (Y-27632)	**44.7 ± 2.6**	**41.8 ± 4.6**	**−49.36**	**−49.82**

The bold values represent the top active compounds.

*The results are expressed as mean values ± SD ($n = 4$). Final concentration of the compounds used in this assay was 100 μM, while final concentration of Y-27632 was 1.6 μM.

**Scores expressed as free energy of binding ΔG in kcal/mol (S) calculated by CLC drug discovery work bench 3.0 for the tested compounds.

***Nil-No enzyme inhibition.

3. Conclusion

In our ongoing research to discover natural Rho-kinase inhibitors, the ethanol extract of the traditional medicine adlay seeds together with 28 compounds, have been assayed for their Rho-kinase (ROCK I and II) inhibitory activity. The results of the study suggest a possible interference of Rho-kinase inhibitory activity to the multiple biological activities of adlay seeds. Our study furthermore, provides a rationale for the folkloric uses of the seeds. The results obtained with the tested compounds could be used for further optimisation and development of natural Rho-kinase inhibitors.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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