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Synthesis and Nrf2 Activating Ability of Thiourea and Vinyl Sulfoxide Derivatives

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Thiourea and vinyl sulfoxide derivatives were designed based on the structures of sulforaphane and gallic acid, prepared and tested for HO-1 inducing activity as a measure of Nrf2 activation, and inhibitory effect on NO production as a measure of anti-inflammatory activity. Both series of compounds showed moderate activity on HO-1 induction, and no inhibitory effect on NO production. Interestingly the thiourea compound **6d** showed better HO-1 induction (71% SFN) than the corresponding isothiocyanate compound **6a** (55% SFN). Overall, it seemed that more efficient electrophile is needed to get more effective Nrf2 activator.

Key Words : Thiourea, Vinyl sulfoxide, Nrf2 activator, Parkinson's disease, Oxidative stress

Introduction

Parkinson's disease is believed to be the outcome of dopaminergic neuronal loss caused by reactive oxidizing species produced by the oxidative stress in substantia nigra of the brain.[1] Dopamine quinone is a reactive oxidizing species that is responsible for such neuronal loss, and, therefore, scavenging the quinone might be a way to prevent dopaminergic neuronal loss from oxidative stress.[2]

Quinone reductase 1 (NQO-1), an enzyme that catalyzes the transformation of quinone to hydroquinone, has been proved to play a crucial role in protecting neuronal cells from cytotoxicity.[3] The induction of NQO-1 depends on Nuclear factor (erythroid-derived 2)-like 2 (Nrf2); on oxidative stress, Nrf2 separates from Keap1 (Kelch-like erythroid associated protein 1) and migrates to the nucleus, where it induces endogenous antioxidant enzymes HO-1 (Heme Oxygenase-1) and NQO-1 by binding to the ARE.

Various compounds including sulforaphane,[4] anethole dithiolethione (ADT),[5] curcumin, and caffeic acid phenethyl ester (CAPE)[6] have been known to protect cell damage through strong activation of Nrf2 (Figure 1). Sulforaphane typically activates Nrf2 by directly forming a covalent bond with the thiol group of cystein residue present in Keap1, and, therefore, might be possible to be used as a neuroprotectant for Parkinson's disease.[7,8]

Previously we were interested in the antioxidant activity of natural products sulforaphane and gallic acid, designed an isothiocyanate compound **KKJC0057** based on their structures, and tested its HO-1 inducing ability as the measure of Nrf2 activation (Figure 1). And also the inhibitory effect on NO production was measured to identify activity against neuroinflammation, which has been known to be an important pathology of Parkinson's disease. **KKJC0057** showed HO-1 inducing ability of 122% compared to sulforaphane at the experimental condition, and good inhibitory activity on NO production (Table 1). We further modified the structure by simplifying the trimethoxyphenyl group of **KKJC0057** to mono-substituted phenyl group and tested their biological activity. The results are also shown in Table 1. The compounds **6a** (R_1=F) and **7a** (R_1=OMe) showed moderate to good HO-1 inducing activity compared to sulforaphane (**6a**: 55%; **7a**: 84%) at the experimental condition. Inhibitory activity on NO production was comparable to **KKJC0057**. Of course the activity of these compounds is

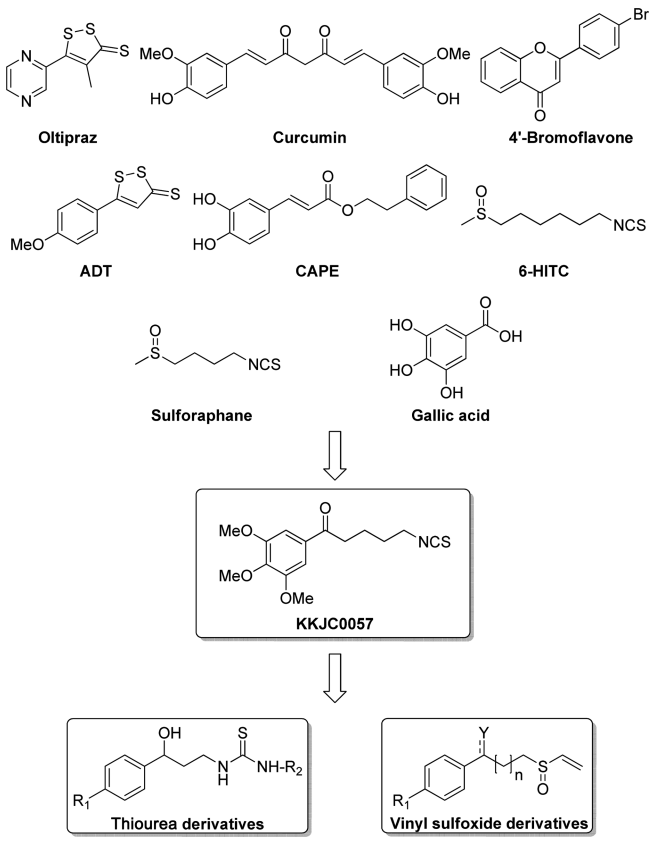
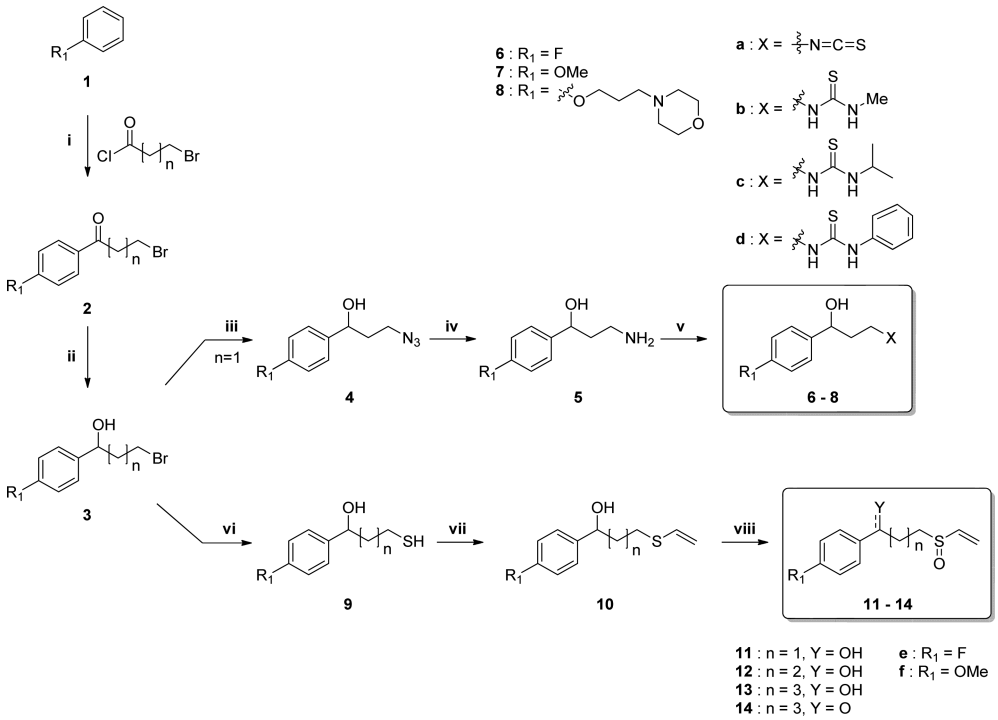


Figure 1. Nrf2 activators and designed novel structures.



Scheme 1. Reagents and conditions: i) $AlCl_3$, DCM, 0 °C to rt, 2 h, 68%; ii) $NaBH_4$, MeOH, 0 °C to rt, 1 h, 90-99%; iii) NaN_3, DMF, 80 °C, 4 h, 76-95%; iv) LAH 1 M in THF, diethyl ether, 0 °C to rt, 1 h, 95%; v) isothiocyanate, DCM, 0 °C to rt, 12 h, 80-99; vi) Potassium ethylxanthate, acetone 0 °C to rt, 12 h and then, LAH 1 M in THF, THF, 0 °C to rt, 12 h, 38-57%; vii) NaH, 1,2-dibromoethane, THF, 0 °C to rt, 1 h, 39-56%; viii) $NaIO_4$, water, 0 °C to rt, 24 h, 30-63%.

supposed primarily to come from the strong electrophile, terminal isothiocyanate group. At this point we were interested in finding some functional groups that can be introduced in place of the isothiocyanate group. Thus we designed the compounds with either thioureido group or vinyl sulfoxide group at the terminus (Figure 1). Thioureido group is actually the product form resulting from the reaction of isothiocyanate and a nitrogen nucleophile; by introducing it we wanted to know how important the isothiocyanate group was. The other substitute, vinly sulfoxide, was chosen with a hope that the vinyl group would act as a nucleophile acceptor.

Results and Discussion

The synthesis of thiourea and vinyl sulfoxide derivatives is shown in Scheme 1. Substituted benzenes **1** were transformed to bromoalkylcarbonyl benzenes **2** *via* Friedel-Crafts acylation reaction with bromoalkylcarbonyl chlorides. The acyl benzenes **2** were then reduced by sodium borohydride to yield secondary alcohol compounds **3**. To prepare thiourea derivatives, terminal bromide of the compounds **3** was substituted by azide to give the compounds **4**, which was then reduced by lithium aluminium hydride to give the amine compounds **5**. By reacting with the corresponding isothiocyanates, the amine compounds **5** could be transformed to thiourea derivatives **6-8** in good yields.[9,10]

Vinyl sulfoxide derivatives were also prepared from the bromides **3**. The bromides **3** were reacted with potassium ethylxanthate followed by reduction with lithium aluminium hydride to give the thiol compounds **9** in moderate yields. The terminal thiol group in the compounds **9** was then vinylated by bromoalkylation and subsequent elimination

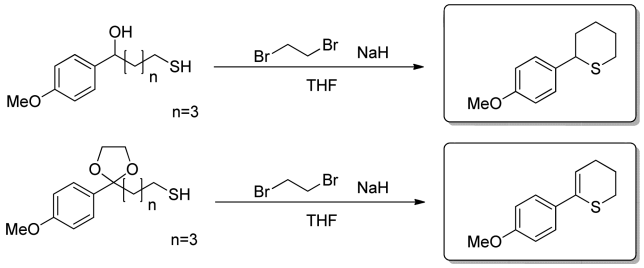
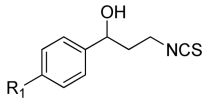


Figure 2. Tetra(Di)hydrothiopyrans formation as side products.

Table 1. Biological activity of isothiocyanate derivatives



6a, 7a

Compounds	R_1	HO-1[a] (%SFN)	NO[b] (%LPS)
KKJC0057		122.6	16
6a	F	54.8 ± 10.0	39.7 ± 2.8
7a	OMe	83.9 ± 1.6	9.8 ± 0.5

[a]Cell: BV2 microglial cell, 2×10^4 cell/Drug treatment time: 24 h. Treat concentration: SFN (5 μM), Compounds (20 μM). [b]NO = Nitric oxide (% LPS, 20 μM). LPS = Lipopolysaccharide

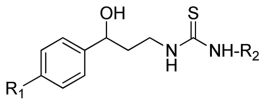
reaction to yield the vinyl sulfides **10**. When n = 3, under the vinylation condition, side reaction of ring formation predominated to yield tetrahydrothiopyran as the major product (Figure 2). Introduction of ketal at the hydroxyl position did not alter the reaction pathway. Thus the vinyl sulfides **10**, when n = 2, could be obtained as minor products. Oxidation of the vinyl sulfides **10** with sodium periodate gave the vinyl sulfoxide derivatives **11-13** as diastereomeric mixtures.[11] The compound **14** was prepared from the ketone **2** *via* thiolation, vinylation and oxidation steps described above.

To know the Nrf2 activating ability of the synthesized compounds, HO-1 induction effect was measured by enzyme-linked immunosorbent assay (ELISA) method.[12] Inhibitory effect on NO production was evaluated by Griess assay method using microglia activated with lipopolysaccharide (LPS).[13]

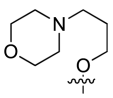
Biological activity of thiourea derivatives are shown in Table 2. Surprisingly the HO-1 induction activity of the thiourea derivatives **6** (R_1=F), was not much different from the corresponding isothiocyanate compound **6a**. Even the compound **6d** (R_2=Ph) showed better HO-1 induction than the compound **6a**. This means that the role of electrophilic group in the series is not so great. In case of the compounds **7** (R_1=OMe), however, the HO-1 inducing activity decreased. Whether the difference in HO-1 inducing effect came from the substituent R_1 or not is not clear. Further study should be needed. The compounds **8** having more water soluble form also showed moderate HO-1 inducing effect. All the synthesized thiourea derivatives did not inhibit NO production.

We expected that the vinyl sulfoxide series, which has a vinyl group with electron accepting capability, would show better biological activity than thiourea derivatives. But they showed moderate HO-1 inducing effect (51-70%) and no inhibition on NO production, similarly to the thiourea

Table 2. Biological activity of thiourea derivatives

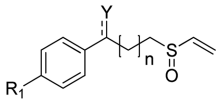


6-8

Compounds	R_1	R_2	HO-1[a] (%SFN)	NO[b] (%LPS)
6b	F	Me	59.2 ± 8.8	123.6 ± 2.3
6c		iPr	39.4 ± 8.2	121.1 ± 10.1
6d		Ph	70.6 ± 7.7	120.5 ± 8.5
7b	OMe	Me	50.8 ± 4.4	124.0 ± 9.6
7c		iPr	59.8 ± 5.1	116.2 ± 8.0
7d		Ph	50.1 ± 3.4	123.6 ± 6.1
8b		Me	53.0 ± 3.5	104.5 ± 5.3
8c		iPr	35.9 ± 5.4	105.2 ± 6.6
8d		Ph	58.5 ± 5.1	95.3 ± 5.3

[a]Cell: BV2 microglial cell, 2 × 10^4 cell/Drug treatment time: 24 h. Treat concentration: SFN (5 μM), Compounds (20 μM). [b]NO = Nitric oxide (% LPS, 20 μM). LPS = Lipopolysaccharide

Table 3. Biological activity of vinyl sulfoxide derivatives



11-14

Compounds	n	Y	R_1	HO-1[a] (%SFN)	NO[b] (%LPS)
11e	1	OH	F	51.9 ± 4.7	106.0 ± 9.7
11f		OH	OMe	69.8 ± 3.7	115.3 ± 8.3
12e	2	OH	F	62.4 ± 2.7	112.3 ± 11.8
12f		OH	OMe	58.0 ± 7.4	101.7 ± 5.7
13e	3	OH	F	54.5 ± 4.1	100.8 ± 10.4
14e		O	F	54.8 ± 1.7	91.7 ± 8.0
14f		O	OMe	51.3 ± 1.5	105.1 ± 15.2

[a]Cell: BV2 microglial cell, 2 × 10^4 cell/Drug treatment time: 24 h. Treat concentration: SFN (5 μM), Compounds (20 μM). [b]NO = Nitric oxide (% LPS, 20 μM). LPS = Lipopolysaccharide

derivatives (Table 3). The effect of chain length was not evident, but the compounds **13** and **14** (n = 3) seemed to be less effective in HO-1 induction than the compounds **11** (n = 1) and **12** (n = 2). Comparing **13e** (Y=OH) and **14e** (Y=O), hydroxy form and keto form showed same effect on HO-1 induction. The effect of the substituent R_1 was not clear.

Conclusion

Thiourea and vinyl sulfoxide derivatives were prepared and evaluated for biological activity to know their possibility of being lead compounds as therapeutics for Parkinson's disease. Both series of compounds showed moderate HO-1 induction activity, but did not inhibit NO production at all. This seemed to come from the absence of effective electrophilic group in the molecules. To find promising leads, compounds with the nucleophile acceptor of intermediate strength between the above mentioned functionalities and isothiocyanate will be investigated.

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Supporting Information. ^{1}H and ^{13}C NMR spectra of the compounds.

References

1. Graumann, R.; Paris, I.; Martinez-Alvarado, P.; Rumanque, P.; Perez-Pastene, C.; Cardenas, S. P.; Marin, P.; Diaz-Grea, F.; Caviedes, R.; Caviedes, P.; Segura-Aguilar, J. *Pol. J. Phar-macol.* **2002**, *54*, 573.
2. Drukarch, B.; Muiswinkel, F. L. *Biochem. Pharmacol*. **2000**, *59*, 1023.
3. Han, J. M.; Lee, Y. J.; Lee, S. Y.; Kim, E. M.; Moon, Y.; Kim, H. W.; Hwang, O. *J. Pharmacol. Exp. Ther.* **2007**, *321*, 249.

4. Mcmahon, M.; Itoh, K.; Yamamoto, M.; Hayes, J. D. *J. Biol. Chem.* **2003**, *278*, 21592.
5. Kensler, T. W.; Qian, G. S.; Chen, J. G.; Groopman, J. D. *Nature Rev. Cancer.* **2003**, *3*, 321.
6. Balogun, E.; Hoque, M.; Gong, P.; Killeen, E.; Green, C. J.; Foresti, R.; Alam, J.; Motterlini, R. *Biochem. J.* **2003**, *371*, 887.
7. Lee, J. S.; Surh, Y. J. *Cancer Lett*. **2005**, *224*, 171.
8. Innamorato, N. G.; Rojo, A. I.; García-Yagüe, Á. J.; Yamamoto, M.; Ceballos, M. L.; Cuadrado, A. *J. Immunol*. **2008**, *181*, 680.
9. Prabhu, K. R.; Pillarsetty, N.; Gali, H.; Katti, K. V. *J. Am. Chem. Soc*. **2000**, *122*, 1554.
10. Custelcean, R.; Gorbunova, M. G.; Bonnesen, P. V. *Chem. Eur. J.* **2005**, *11*, 1459.
11. Ruano, J. L. G.; Vega, J. M. G.; Parellada, M. D.; Secundino, M. A. *J. Chem. Soc. Perkin Trans. II* **1988**, 1573.
12. Kitchin, K. T.; Anderson, W. L.; Suematsu, M. *J. Immunol. Methods*. **2001**, *247*, 153.
13. Sun, J.; Zhang, X.; Broderick, M.; Fein, H. *Sensors* **2003**, *3*, 276.