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Discovery, Total Synthesis, HRV 3C-Protease Inhibitory Activity, and Structure–Activity Relationships of 2-Methoxystypandrone and Its Analogues

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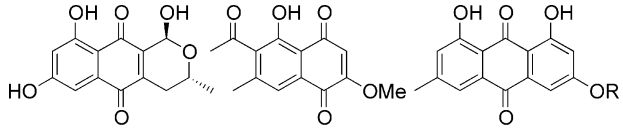
Abstract—2-Methoxystypandrone, a naphthoquinone, was isolated from a Chinese herb *Polygonum cuspidatum* by bioassay guided fractionation using HRV 3C-protease assay. It showed an IC_{50} value of 4.6 μM and is moderately selective. A new 10-step, total synthesis of 2-methoxystypandrone was accomplished in 45% overall yield using a Diels–Alder approach. Several analogues of this compound were prepared. Isolation, synthesis and HRV 3C-protease structure–activity relationships of these compounds have been described.

Human rhinoviruses (HRVs) are members of the picornavirus family of viruses and are the single most significant causative agents of the common cold.[1] More than 100 serotypes of these viruses are known and they typically infect upper respiratory tract in humans and target nasal epithelial cells. A 220 kDa polypeptide is translated by the positive-sense viral RNA genome upon entry into the host cell. This polypeptide is proteolytically processed by virus-specific proteases (HRV 2A and HRV 3C) to afford structural and functional proteins that are required for replication of HRV.[2] HRV 3C-protease, a cysteine protease, is responsible for the polypeptide cleavage between Q-G amino acids and plays a critical role in the replication cycle of HRVs and thus constitutes a potential therapeutic target for the control of HRVs and common cold.[2,3] AG-7088, one of the inhibitors of this enzyme is in phase II clinical development for the treatment against the infections of HRVs.[4]

Screening of natural product extracts against HRV 3C-protease using a small peptide containing Q-G scissile bond as a substrate led to the discovery of thysanone (**1**)[5] as a novel inhibitor from an extract of a fungus, *Thysanophora penicilloides*. Continued screening revealed potent HRV 3C-protease activity in the extracts of Chinese herb, *Polygonum cuspidatum*. The bioassay guided fractionation of the extract led to the isolation of three compounds identified as 2-methoxystypandrone (**2**), emodin (**3**), and methoxyemodin (**4**). The compound **2** showed IC_{50} value of 4.6 μM in HRV 3C-protease assay. The present paper describes the discovery, structure, a new total synthesis, HRV 3C-protease inhibitory activity, and SAR of 2-methoxystypandrone and related compounds.



Isolation and Structure

Sephadex LH 20 chromatography of a 1:1 CH_3OH–CH_2Cl_2 extract (100 mg) of *P. cuspidatum* followed by chromatographies on silica gel and reversed-phase C-8 HPLC yielded **2** (1 mg), **3** (5 mg), and **4** (2 mg). The structure of **2** was elucidated as 2-methoxystypandrone by comparison of spectral data (UV, IR, and ^1H NMR).[6] The structures of **3** and **4** were determined by comparison with authentic samples.

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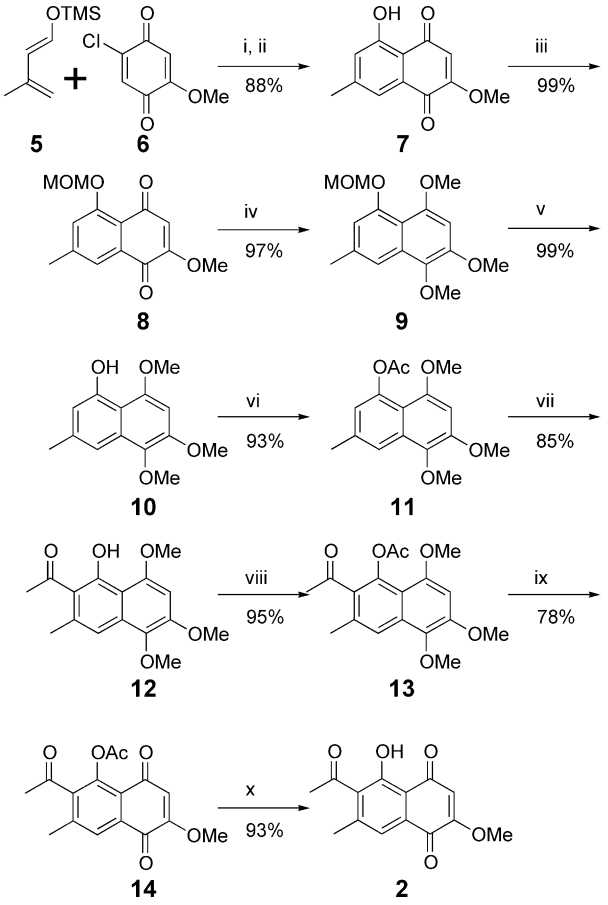
Synthesis of 2-Methoxystypandrone

The quantity of 2-methoxystypandrone isolated from the plant extract was very small and resupply of the compound for further biological evaluations had to be accomplished by a total synthesis. A 14-step total 9% overall yielding synthesis of this compound was reported by Hughes and Sargent.[6b] However, we decided to design a new synthesis that would be more amenable for analogue synthesis for SAR. In our synthetic approach we took advantage of the regio-selective Diels–Alder reactions[7] of suitably substituted chloro-benzoquinone and appropriately substituted *O*-silyloxy butadiene followed by oxidation for the construction of the naphthoquinone. A 10-step 45% overall yielding synthesis (Scheme 1) of **2** follows.

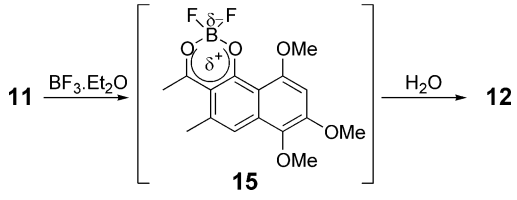
Thus, Diels–Alder reaction of 1-trimethylsilyloxy-1, 3-butadiene[8] (**5**) with 2-methoxy-5-chloro-benzoquinone (**6**) in refluxing benzene followed by Jones oxidation afforded naphthoquinone (**7**) in 88% yield. MOM protection of the phenolic group produced compound **8** in 99% yield, which was reduced with sodium dithionite and alkylated with dimethyl sulfate to give trimethyl ether (**9**) in 97% yield. Deprotection of the MOM ether turned out to be challenging but was efficiently accomplished by using a mixture of dioxane–MeOH–H_2O–H_2SO_4 (3:6:2:0.8), which gave deprotected product **10** in 99% yield after chromatography. Standard acetylation of compound **10** furnished compound **11** in 93% yield. Fries rearrangement of compound **11** with $BF_3{\cdot}Et_2O$ at 60 °C exclusively produced *ortho*-acetylated product **12** in 85% yield (vide infra), which was acetylated to give acetate **13** in 95% yield. Oxidation of compound **13** with ceric ammonium nitrate (CAN) in a mixture of CH_3CN–H_2O yielded[9] 78% of *para*-quinone **14**, which upon saponification furnished **2** which was identical (TLC, HPLC, ^1H NMR) to natural product 2-methoxystypandrone.



Scheme 1. Reagents and conditions: (i) Benzene, reflux, 24 h; (ii) Jones oxidation; rt, 20 min; (iii) MOMCl, DIPEA, CH_2Cl_2, 0 °C to rt, 16 h; (iv) (a) Sodium dithionite, dioxane–H_2O, 1:1, rt, 15 min; (b) KOH, dimethyl sulfate, rt, 2 h; (v) dioxane–MeOH–H_2O–H_2SO_4, 3:6:2:0.8, rt, 16 h; (vi) Ac_2O, C_5H_5N, rt, 20 h; (vii) $BF_3{\cdot}Et_2O$, 60 °C, 35 min; (viii) Ac_2O, C_5H_5N, rt, 20 h; (ix) CAN (excess), CH_3CN–H_2O, 3:1, 30 min; (x) KOH, MeOH–H_2O, 6:1, 0.5 min, 6 N HCl.

Fries Rearrangement

$BF_3{\cdot}Et_2O$ catalyzed Fries rearrangement of compound **11** produced stable BF_2-adduct **15** (Scheme 2) which was purified by silica gel chromatography and was characterized by spectral analysis.[10] It showed a molecular ion at *m*/*z* 338 (with *m*/*z* 337, which was 20% of *m*/*z* 338 peak). The ^{11}B NMR spectrum showed one signal for the boron nucleus at δ 0.20 (relative to external reference of $BF_3{\cdot}Et_2O$ at δ 0.0). The ^{19}F NMR spectrum of **15** showed two signals for equivalent fluorine atoms at δ −147.52 and δ −147.59 (relative to internal C_6F_6 at δ −163.0) in a 1:4 ratio consistent with the natural abundance ratios of ^{10}B and ^{11}B, respectively. In addition, the broad band decoupled ^{13}C NMR spectrum of **15** showed two triplets at δ 193.9 (J = 1.7 Hz) and δ 171.1 (J = 2.2 Hz) due to covalent bonding of the boron atom to CH_3CO and the aromatic oxygen. The hydrolysis of the adduct in water was slower (45% conversion in 30 min and 100% after overnight as observed by ^1H NMR analysis in CD_2Cl_2 and addition of D_2O) and was fast in MeOH.

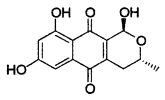
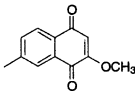
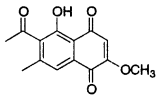
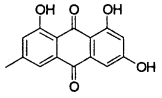
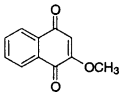
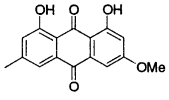
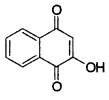
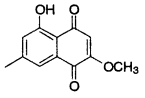
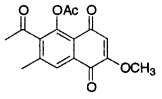
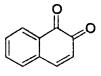
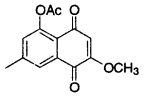
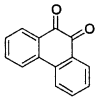
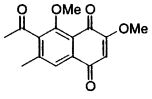
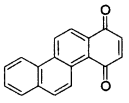
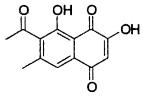
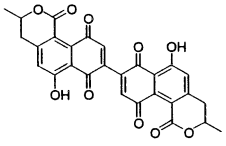


Scheme 2.

Sources of Analogues

Compound **16** (Table 1) was prepared by standard acetylation of compound **7**. Compounds **17** and **18** were prepared by a method described by Jung et al.[11] Compound **19** was isolated as an occasional by-product of the Jones oxidation reaction during preparation of **7**. 5-Hydroxy-7-methyl-1,4-naphthoquinone (**20**) was prepared by analogous cycloaddition reactions of **5** with 2-chloroquinone followed by oxidation. Compounds **21**–**26** were acquired from Aldrich Chemical Company. Xanthomegnin (**27**) was obtained from our internal collections.

Table 1. List of compounds and biological activities

Compd	Structure	HRV3C (IC_{50}, μM)	Papain (IC_{50}, μM)	Compd	Structure	HRV3C (IC_{50}, μM)	Papain (IC_{50}, μM)
1		47	NT	**19**		10.5	NT
2		4.6	23	**20**		6.4	NT
3		> 150	NT	**21**		26	NT
4		> 150	NT	**22**		> 150	NT
7		2.4	NT	**23**		21	76% (29 μM)
14		8.1	33	**24**		8.2	67% (120 μM)
16		40	NT	**25**		1.4	24% (91 μM)
17		124	NT	**26**		46.5	45% (18 μM)
18		> 150	NT	**27**		6	NT

NT, not tested.

Structure–Activity Relationship

2-Methoxystypandrone (**2**) was evaluated in HRV 3C-protease HPLC assay.[2a] It showed an IC_{50} value of 4.6 μM and the inhibition was time dependent. In order to measure the selectivity of this compound against other cysteine proteases, it was evaluated against papain. It showed 5-fold selectivity for HRV 3C-protease over papain (Table 1). Naphthoquinone without acetyl substitution (cf. **7**) was 2-fold more potent than the corresponding acetylated compound **2**. Acetylation of the phenolic group caused 2-fold reduction (cf. **14**) compared to the parent compound **2** and over 16-fold reduction (cf. **16**) compared to compound **7**. Elimination of either phenolic (**19**) or methoxy (cf. **20**) groups from **7** caused 5- or 3-fold diminution of the HRV 3C-protease activity. Elimination of both the methyl and the hydroxy groups of **7** caused more significant reduction in HRV 3C-protease activity (cf. **21**) but the elimination of methoxy group (cf. **23**), the third substituent, did not have any deleterious effect on the potency (26 vs 21 μM). However, it appears that the elimination of all three substituents (cf. **23**) led to the elimination of the selectivity for HRV 3C-protease. The transposition of the methoxy group from C-2 to C-3 caused significant reduction in the activity (**14** vs **17**). Complete loss of the activity was observed when the methoxy group was replaced with a hydroxy group (**21** vs **22**) and (**14**, **17** vs **18**). The *ortho*-quinones were generally more potent than corresponding *para*-quinones (**23** vs **24**). The size of the quinones has little effect on the protease activity. For example, complex tricyclic naphthoquinones such as thysanone (**1**) and xanthomegnin (**27**) showed IC_{50} values of 47 and 6 μM, respectively. Substituted 1,4-anthraquinones such as emodin (**3**) and methylemodin (**4**) were inactive. In contrast the simple 9,10-phenathraquinone was the most active compound of the series and showed an IC_{50} value of 1.4 μM with a high degree of selectivity. Chrysenequinone (**26**) showed a modest activity (IC_{50} 46.5 μM). The activity profiles of 1,4-quinones (cf. **2**) and 1,2-qunones (cf. **25**) were different. The former compound showed a time dependent inhibition which was not affected by pre-incubation reaction with DTT whereas the latter is

a time dependent inhibitor but inhibition is potentiated by reaction with DTT.

2-Methoxystypandrone and other active compounds reported in this letter contain reactive sites, which is consistent with most of the inhibitors of HRV 3C-protease reported in the literature that also possess the Michael addition site including clinical candidate AG-7088.[3,4] The mode of action of these inhibitors are through irreversible inactivation of the enzyme.

In summary, we have described the discovery, efficient synthesis and HRV 3C-protease activity of 2-methoxystypandrone and analogues. In addition, we also described and compared the activities of *ortho*- versus *para*-quinones. It appears that the mode of action of the two classes of compounds is different.

Acknowledgements

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References and Notes

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9. Experimental details. Aqueous solution of CAN (300 mg in 0.4 mL H_2O) was added drop wise (in 1 h) to a CH_3CN solution of **13** (50 mg in 0.4 mL CH_3CN). After 3 h stirring at rt the reaction mixture was diluted with 10 mL H_2O, extracted with CH_2Cl_2 and chromatographed on silica gel column. Elution with 49:1 CH_2Cl_2–MeOH afforded 35 mg of **14** as a chromatographically homogeneous yellow powder.
10. **15**: HREIMS (*m*/*z*) 338.1143 (calcd for $C_{16}H_{17}O_5BF_2$:338.1136), ^{1}H NMR (400 MHz, CD_2Cl_2) δ, 2.60 (3H, d, $J=1.9$ Hz), 2.79 (3H, s), 3.82 (3H, s), 4.02 (3H, s), 4.03 (3H, s), 6.58 (1H, s), 7.26 (1H, m), ^{13}C NMR (100 MHz, CD_2Cl_2) 25.1, 28.3, 56.7, 57.1, 61.6, 95.6, 109.3, 114.4, 117.3, 134.4, 136.1, 136.7, 158.2, 160.7, 171.1 (t, $J=2.2$ Hz), 193.9 (t, $J=1.8$ Hz). **12**: ^{1}H NMR (250 MHz, CD_2Cl_2) δ, 2.34 (3H, d, $J=0.8$ Hz), 2.56 (3H, s), 3.84 (3H, s), 3.97 (3H, s), 4.05 (3H, s), 6.60 (1H, s), 7.31 (1H, t, $J=0.8$ Hz), 9.78 (1H, br s).
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