Efficient, collective synthesis and nitric oxide inhibitory activity of rubrolides E, F, R, S and their derivatives

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Graphical Abstract

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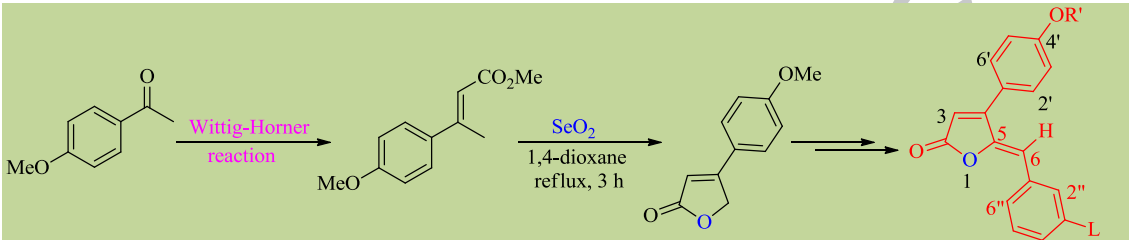

- Unified synthetic strategy
- 14-48.5% overall yields
- Potent in vitro nitric oxide (NO) inhibition
- No cytotoxicity
- IC_{50} (μM): **1e**: 10.53; **1ea**: 13.01; **1f**: 8.53; **1fa**: 11.91; **1r**: 12.51; **1s**: 17.85; **1sa**: 13.29

Rubrolide E (**1e**): R = R' = L = H
Rubrolide E derivative (**1ea**): R = R' = Me, L = H
Rubrolide F (**1f**): R = Me. L = R' = H
3"-Bromo rubrolide F (**1fa**): R = Me. R' = H, L = Br
Rubrolide R (**1r**): R = R' = H, L = -CH_2-CH=C(CMe)$_2$
Rubrolide S (**1s**): R-L = -C(CMe)$_2$-CH_2-CH_2-, R' = H
Rubrolide S derivative (**1sa**): R-L = -C(CMe)$_2$-CH=CH-, R' = H

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ABSTRACT

An efficient first synthesis of biologically significant natural butenolides, rubrolides F (**1f**), R (**1r**), S (**1s**) & its 7",8"-didehydro derivative (**1sa**), and 3"-bromo rubrolide (**1fa**) along with the synthesis of rubrolide E (**1e**) and its di-*O*-methyl derivative (**1ea**) is accomplished in a collective fashion from commercially available and inexpensive precursors in overall yields of 14-48.5%. Key features are Wittig-Horner reaction, SeO_2-induced tandem allylic hydroxylation/intramolecular cyclization and Knoevenagel condensation. Next, in their inhibitory activity towards nitric oxide (NO) production in lipopolysaccharide-induced RAW 264.7 macrophages as an indicator of *anti*-inflammatory activity, all compounds displayed good inhibitory activity in a concentration-dependent manner. None of the compound exhibited notable cytotoxicity at the highest concentration (10 μM) and IC_{50} values are found in the range from 8.53 to 17.85 μM.

Butenolide, a five membered α,β-unsaturated γ-lactone scaffold, is found in a plethora of natural products and pharmaceutical agents.[1] The structural discrepancy within the butenolides is complemented by various biological activities. Rubrolides are biologically active marine ascidian metabolites belong to the family of butenolides with an alkylidene and phenolic moieties appendages at C-5 and C-4 position, respectively.

Rubrolides E (**1e**) and F (**1f**) were first isolated from a marine tunicate *Ritterella rubra* along with rubrolides A-D, G, H and possessed strong antibacterial as well as selective protein phosphatases 1 and 2A inhibition activity.[2] Recently, rubrolide R (**1r**) and S (**1s**) have been isolated from the fermentation broth of the marine-derived fungus *Aspergillus terreus* OUCMDZ-1925.[3] Of note, rubrolide R (**1r**) was shown to display comparable or superior antioxidant activity to those of trolox and ascorbic acid with an IC_{50} value of 1.33 mM while rubrolide S (**1s**) exhibited comparable or superior anti-influenza A (H1N1) virus activity to that of ribavirin with an IC_{50} value of 87.1 mM.[3] Halogenated rubrolides such as 3"-bromorubrolide (**1fa**) (Fig. 1) were also isolated from *Synoicum globosum* ascidian and showed antibacterial activity.[4] Other promising activities of rubrolides and their analogues include inhibition of bacterial biofilm formation,[5] anticancer,[6] human aldose reductase (ALR2) inhibition,[7] anti-tobacco mosaic virus (anti-TMV) activity[8] and inhibition of photosynthesis.[9]

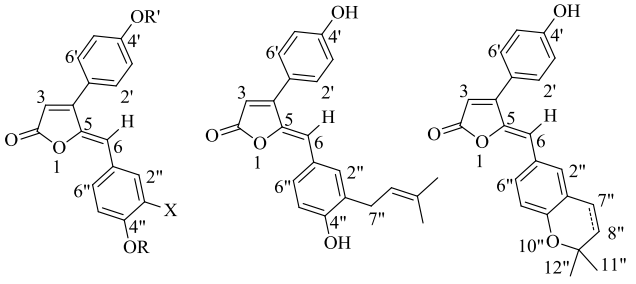

Figure 1: Structures of rubrolides E (**1e**), F (**1f**), R (**1r**) and S (**1s**), 3"-bromo rubrolide (**1fa**) and their derivatives (**1ea** and **1sa**).

The uncomplicated molecular architecture in conjunction with multifarious biological activities of rubrolides have attracted the endeavours from synthetic community and, to date, syntheses of rubrolides C, E, L and M have been achieved[10] and some groups have also made great efforts in their analogues preparation.[11] Recently, Barbosa and co-workers disclosed a concise synthesis of rubrolides B, I, K and O using Suzuki coupling and a late-stage regioselective bromination reaction as key steps.[12]

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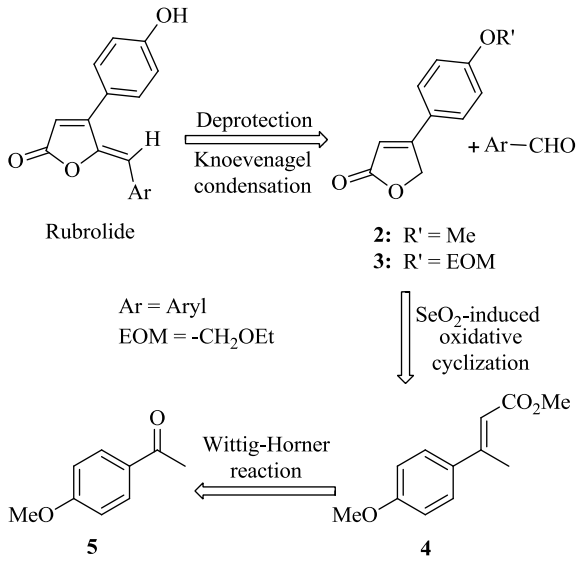


Scheme1: General retrosynthetic analysis of rubrolides under the current study.

In connection with our continued interest[13] in the synthesis of bioactive natural products and their analogues, herein, we describe the first synthesis of rubrolides F (**1f**), R (**1r**), S (**1s**) & its 7",8"-didehydro derivative (**1sa**), and 3"-bromo rubrolide (**1fa**) along with the synthesis of rubrolide E (**1e**) and its di-*O*-methyl derivative (**1ea**). We have envisioned that the target compounds can be achieved by a unified synthetic sequence comprising Wittig-Horner reaction, SeO_2-induced tandem oxidative cyclization and Knoevenagel condensation as pivotal steps (Scheme 1).

As shown in Scheme 2, our synthetic sequence commenced with the Wittig-Horner olefination of commercially available 4-methoxyacetophenone (**5**). Reaction of **5** with trimethyl phosphonoacetate in the presence of sodium hydride (NaH) at 0 ºC, gave the corresponding *E*-isomer of α,β-unsaturated ester **4** in 83% yield. Ester **4** was subjected to selenium dioxide (SeO_2)-oxidation, wherein, the allylic methyl group underwent hydroxylation and subsequent intramolecular cyclization furnished rubrolides back bone, 4-(4-hydroxyphenyl)furan-2(5*H*)-one (**2**). Treatment of **4** with 1.5 equiv. of SeO_2 in 1,4-dioxane under reflux condition for 3 h offered the compound **2** in 73% along with the corresponding over oxidised product, aldehyde **6**, in 13% as a *E*,*Z*-mixture. Varying the time and equivalents of SeO_2 resulted in the diminished yields of **2** due to either remaining starting material or it's over oxidation. Previously, Kagabu and co-workers reported the similar strategy with SeO_2 in acetic acid and perchloric acid as a catalyst, wherein, the reaction times are relatively longer and had tedious work up procedure.[14] Demethylation of compound **2** using BBr_3 produced compound **7** in 97% yield. Compound **7** was protected as 4'-ethoxymethyl (EOM) ether **3** (91%) using chloromethyl ethyl ether (EOM-Cl), K_2CO_3 and catalytic tetrabutylammonium iodide (TBAI).

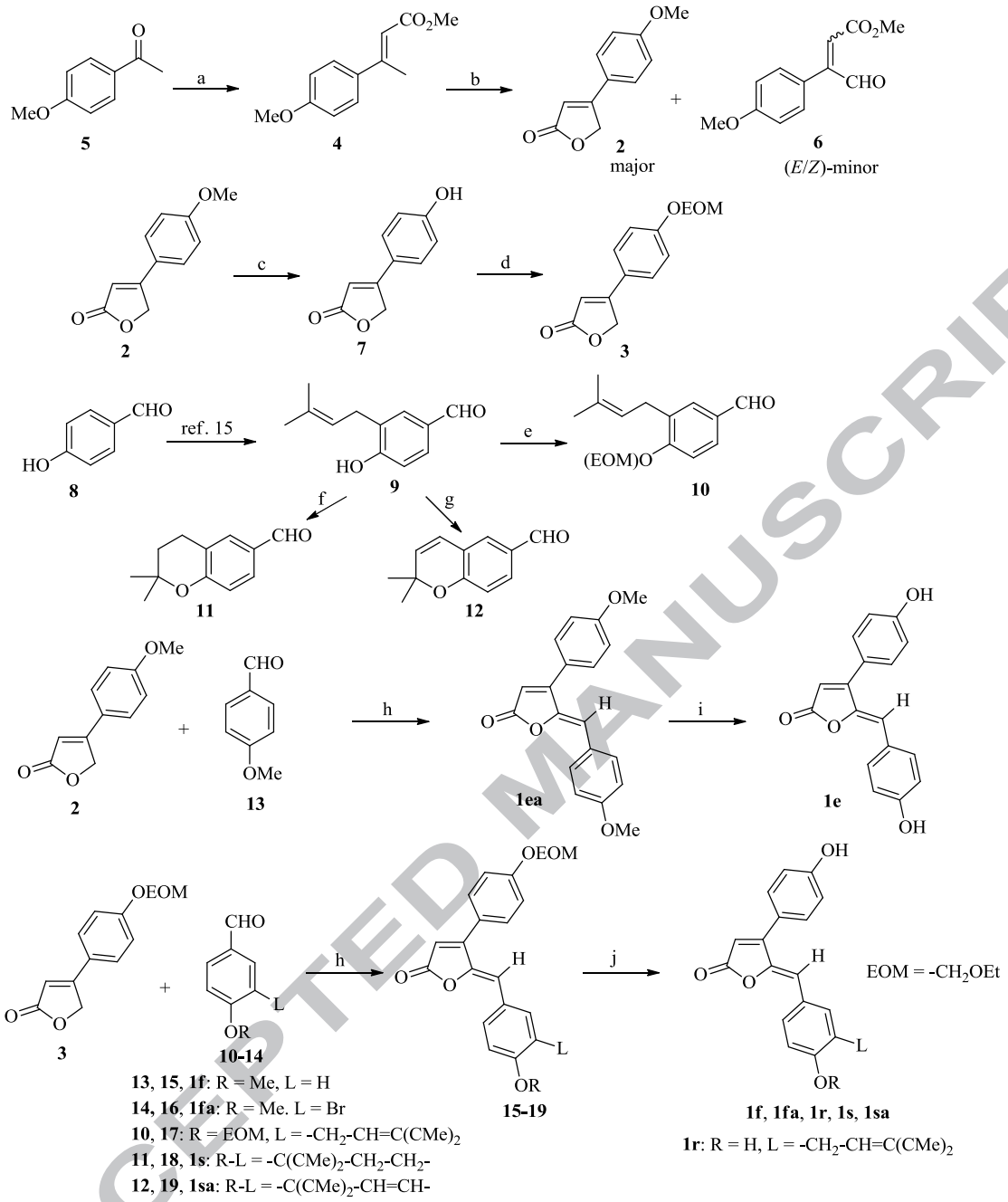
With requisite key intermediates **2** and **3** in hand, we then turned our attention to procure the corresponding aldehydes. Dimethylallylation of OH group of 4-hydroxybenzaldehyde (**8**) with *tert*-butyl (2-methylbut-3-en-2-yl) carbonate and catalytic tetrakis(triphenylphosphine)palladium(0) [$Pd(PPh_3)_4$] followed by microwave-promoted Claisen rearrangement of the resulting ether afforded C-prenylated product **9** in 80% yield.[15] The free phenolic group of **9** was protected as a EOM ether by reacting with EOM-Cl and K_2CO_3/TBAI system to afford compound **10**. Cyclization of **9** using catalytic *p*-toluenesulfonic acid monohydrate (*p*-TsOH·H_2O) gave **11** in 90% yield. Next, 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ)-promoted oxidative cyclization of **9** in refluxing benzene resulted the chromene aldehyde **12** in 81% yield.

To complete the synthesis of the butenolide natural products, rubrolides, with **2**, **3** and aldehydes **10**-**14**, we chose a two-step reaction sequence comprising the Knoevenagel condensation and deprotection. Compound **2** underwent Knoevenagel condensation with *p*-anisaldehyde (**13**) in the presence of piperidine as a base in MeOH to give butenolide **1ea**, which was subsequently subjected to demethylation with boron tribromide (BBr_3) to afford rubrolide E (**1e**) in 95% yield. Next, Knoevenagel condensation of **3** with **13**, 3-bromo-4-hydroxybenzaldehyde (**14**) and aldehydes **10**-**12** afforded butenolides **15**-**19** in 51-70% yields, respectively. To our delight, deprotection of EOM-group of compounds **15**-**19** was smoothly proceeded with Dowex® resin and furnished rubrolide F (**1f**), 3"-bromo rubrolide (**1fa**), rubrolides R (**1r**), S (**1s**) and its derivative (**1sa**), respectively. All the natural products (**1e**, **1f**, **1fa**, **1r** and **1s)** and their derivatives (**1ea** and **1sa**) were settled from their spectral (^1H, ^{13}C- NMR and MS) data.

Inhibition of nitric oxide (NO) production:

Inflammation is an essential host response to infection by pathogens, damaged cells or irritants. This complex but highly coordinated process results in the increased production of various soluble mediators including chemokines, cytokines, free radicals (such as nitric oxide (NO)), and eicosanoids (prostaglandins) by resident cells (that is, tissue macrophages, lymphocytes, fibroblasts, endothelial cells and mast cells) in the injured or infected tissue.[16] NO, produced from L-arginine by nitric oxide synthase (endothelial-NOS, neuronal-NOS and inducible-NOS), is one of the important one among the above stated mediators. The role of NO in the pathogenesis of inflammation tightly depends upon its concentration.[17] Physiologically vital amounts of NO produced by inducible-NOS (iNOS) in response to inflammatory stimuli help in mounting an effective defense against pathogens, whereas, overproduction of NO by iNOS could potentially result in tissue damage and activation of proinflammatory mediators associated with acute and chronic inflammations.[18] Hence, more attention is now being paid to the pharmacological interference with the NO production cascade and can be considered as a promising strategy for the development of new *anti*-inflammatory drugs.

To investigate the inhibitory effect of the synthesized rubrolides (**1e**, **1f**, **1r**, **1s** and **1fa**) and their analogues (**1ea** and **1sa**) against NO production, we measured the amount of nitric oxide (NO) in lipopolysaccharide (LPS)-stimulated RAW 264.7 cells as an indicator of *anti*-inflammatory activity, following our previous procedure.[13a] *N*-Monomethyl-L-arginine (L-NMMA) was employed as a positive control.[19] As shown in Table 1, all the compounds had a concentration-dependent inhibitory effect on NO production. At the highest concentration (10 μM), rubrolide F (**1f**) had the strongest inhibitory effect that means low amount of NO release from the cells (45.19 ± 4.91; IC_{50} = 8.53 μM) which was in comparison with the positive control, L-



Scheme 2: Synthesis of rubrolides and derivatives. *Reagents and conditions:* (a) trimethyl phosphonoacetate, NaH, 0 ºC, 0.5 h, then **5**, rt, 14 h, 83%. (b) SeO_2, 1,4-dioxane, reflux, 3 h, 73% (**2**), 13% (**6**). (c) BBr_3 (1.0 M in CH_2Cl_2), -78 0 ºC then rt, 24 h, 97%. (d) EOM-Cl, K_2CO_3, TBAI, 0 ºC then rt, 15 h, 91%. (e) EOM-Cl, K_2CO_3, TBAI, 0 ºC then rt, 20 h, 69%. (f) *p*-TsOH·H_2O, toluene, reflux, 2 h, 90%. (g) DDQ, benzene, reflux, 45 min, 81%. (h) piperidine, MeOH, 25-30 ºC, 15 h, 80% (**1ea**), 69% (**15**), 53% (**16**), 51% (**17**), 70% (**18**), 68% (**19**). (i) BBr_3 (1.0 M in CH_2Cl_2), -78 0 ºC then rt, 24 h, 95%. (j) Dowex® resin, MeOH, 35 ºC, 24 h, 95% (**1f**), 89% (**1fa**), 51% (**1r**), 95% (**1s**), 83% (**1sa**).

NMMA (44.72 ± 1.18; IC_{50} = 5.13 µM) followed by rubrolide S derivative (**1sa**) (53.87 ± 3.17; IC_{50} = 13.29 µM) and rubrolide E (**1e**) (54.47 ± 2.31; IC_{50} = 10.53 µM). Next, cell viability assay was conducted to discern whether the inhibition of NO production resulted from the cytotoxicity of the tested compounds. All the compounds did not have significant cytotoxicity at the highest concentration (10 µM) leading to the effective inhibition of NO production (Table 1). No clear structure-activity relationship was observed in this study, however, our results specified further examples of the importance of rubrolide compounds as significant NO production inhibitors.

In conclusion, we have achieved an efficient first synthesis of bioactive natural butenolides, rubrolides F (**1f**), R (**1r**), S (**1s**) & its 7",8"-didehydro derivative (**1sa**), and 3"-bromo rubrolide (**1fa**) along with synthesis of rubrolide E (**1e**) and its di-*O*-methyl derivative (**1ea**) in a collective manner starting from commercially available and inexpensive precursors and in overall yields of 14-48.5%. This synthetic approach relies on the application of Wittig-Horner reaction and SeO_2-induced tandem oxidative cyclization, which efficiently provides an access to the rubrolide core skeleton. Additionally, synthesized rubrolides screened for their inhibitory effect against NO production in LPS-induced RAW 264.7 cells, in which, all compounds showed

significant inhibition in a concentration-dependent manner with no cytotoxicity. IC_{50} values are found in the range from 8.53 to 17.85 μM. Moreover, this efficient and flexible synthetic route offers opportunities to make analogues of rubrolides and may facilitate their biological studies.

Table 1. NO production inhibitory activities of rubrolides E (**1e**), F (**1f**), R (**1r**), S (**1s**), 3"-bromo rubrolide (**1fa**) and their derivatives (**1ea** and **1sa**)

Compound	NO Production[a,b]		Proliferation[a]		IC_{50} (μmol/L)
	1 μmol/L	10 μmol/L	1 μmol/L	10 μmol/L	
Medium	6.71 ± 1.61	6.71 ± 1.61	100 ± 0.03	100 ± 0.03	
LPS	98.03 ± 2.50	98.03 ± 2.50			
1e	84.99 ± 2.77	54.47 ± 2.31***	103.83 ± 0.07	108.74 ± 0.02	10.53
1ea	87.15 ± 2.16	66.25 ± 0.56***	95.08 ± 0.03	97.27 ± 0.05	13.01
1f	87.95 ± 0.33	45.19 ± 4.91***	100.55 ± 0.08	103.28 ± 0.01	8.53
1fa	86.92 ± 1.55	57.8 ± 1.08***	97.27 ± 0.04	97.27 ± 0.05	11.91
1r	79.61 ± 4.11**	61.58 ± 1.88***	98.91 ± 0.03	97.81 ± 0.05	12.51
1s	90.58 ± 5.67	68.31 ± 2.80***	95.08 ± 0.03	93.99 ± 0.04	17.85
1sa	89.03 ± 0.80	53.87 ± 3.17***	97.81 ± 0.07	92.35 ± 0.05	13.29
L-NMMA	75.16 ± 5.12***	44.72 ± 1.18***	99.64 ± 0.02	95.81 ± 0.06	5.13

[a] The results are reported as mean value ± SEM for n = 3. Statistical significance is based on the difference when compared with LPS-treated groups (**P < 0.01 and ***P < 0.001).

[b] Inhibition is based on LPS.

Supplementary Material

Supplementary data (experimental procedures and characterization data and copies of ^{1}H- and ^{13}C-NMR spectra) associated with this article can be found in the online version at

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Highlights

1. First synthesis of rubrolides F, R, S and their analogues in 14-36% overall yields.
2. Synthesis of rubrolide E has also been achieved with an overall yield of 46%.
3. Key steps: Wittig-Horner, SeO_2-induced oxidative cyclization & Knoevenagel condensation.
4. Significant in vitro nitric oxide production inhibition without notable cytotoxicity.
5. IC_{50} values are found in the range from 8.53 to 17.85 μM.