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Ring-expanding rearrangement of 2-acyl-5-arylidene-3,5-dihydro-*4H*-imidazol-4-ones in synthesis of flutimide analogs

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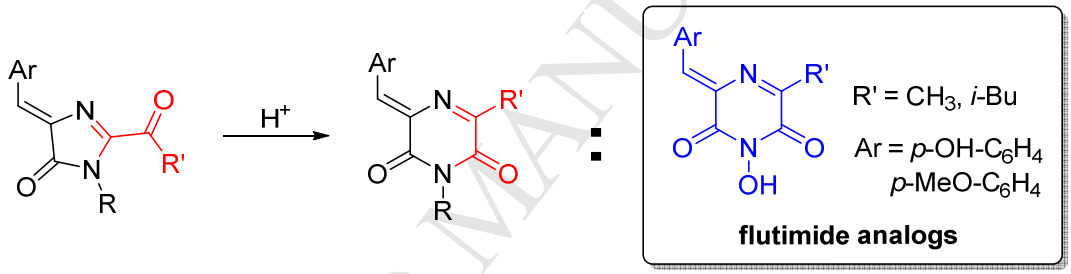
Graphical abstract



Abstract

The RNA-dependent transcriptase of influenza virus is an attractive antiviral target, still not addressed by any commercialized drugs. Flutimide, a fungal metabolite, comprising an unusual 2,6-diketopiperazine core has earlier been shown to inhibit the endonuclease activity of influenza transcriptase. In this paper we present a novel synthetic approach to 2,6-diketopiperazines, based on the rearrangement of 2-acyl-5-arylidene-3,5-dihydro-4*H*-imidazol-4-ones and synthesis and anti-influenza evaluation of a series of novel flutimide analogs.

Key words:

Influenza, flutimide, imidazolones, rearrangement.

1. Introduction

The RNA-dependent transcriptase of influenza virus is an attractive antiviral target, still not addressed by any commercialized drugs. A number of inhibitors for this enzymatic complex have been proposed, including 4-substituted 2,4-dioxobutanoic acids[1] and *N*-hydroxy amides and imides.[2] In 1995 Singh and colleagues reported isolation and structural characterization of a fungal metabolite flutimide, that was shown to selectively inhibit the endonuclease ativity of the influenza viral transcriptase at micromolar concentrations ($IC_{50} \sim 3\mu M$).[3]

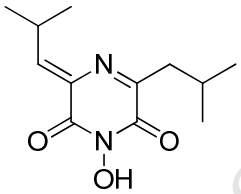


Fig 1. Flutimide, a fungal metabolite possessing an anti-influenza activity

Flutimide was found to have an unusual structure, comprising a 2,6-diketopiperazine core (Fig. 1.). There are only few examples of natural 3,4-dehydro-2,6-diketopiperazines, including antibacterial agent Coelomycin and its derivatives.[4] Several synthetic approaches to this heterocyclic core have been reported.[5]

The total synthesis of flutimide was presented in 1995 by the Singh research group.[6] Later, in 2001 the same team described synthesis of flutimide structural analogs in order to find compounds with improved activity.[5a] Several derivatives showed increased potencies compared to flutimide (down to 0.8 μM), but the method of their synthesis included about 12 stages and provided low total yield.

In this paper we present a novel synthetic approach to 2,6-diketopiperazines, based on the rearrangement of 2-acyl-5-arylidene-3,5-dihydro-4*H*-imidazol-4-ones and synthesis and anti-influenza evaluation of a series of novel flutimide analogs.

2. Results and discussion

In the course of our investigations in the chemistry of chromophores of fluorescent proteins we found that synthetic chromophore of the asFP595 chromoprotein from *Anemonia sulcata* and its analogs easily transform into 2,6-diketopiperazines in acidified aqueous solutions (Fig 2.).[7]

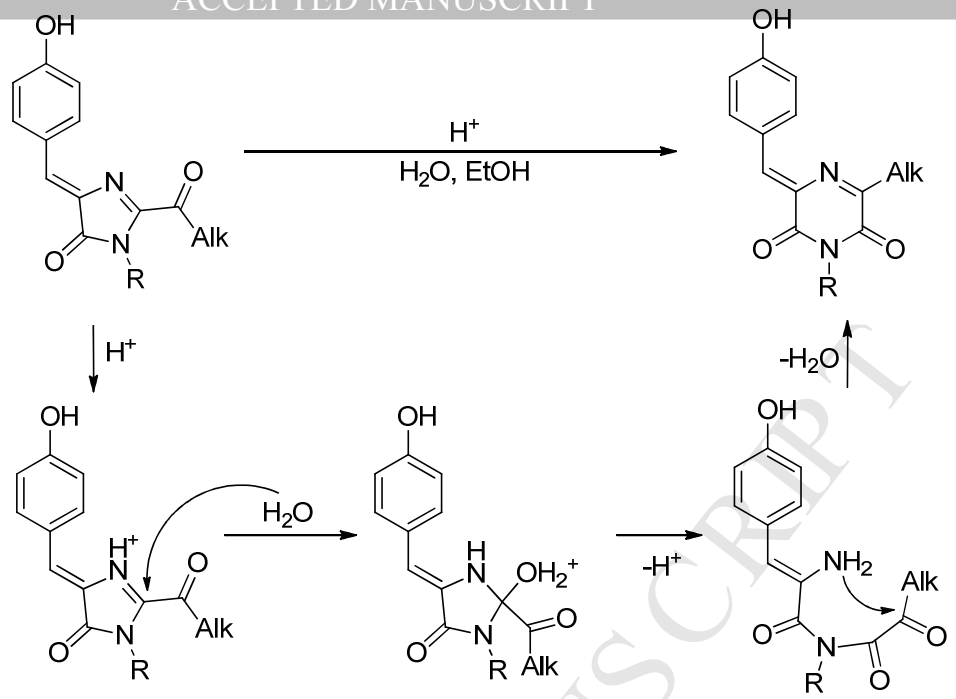
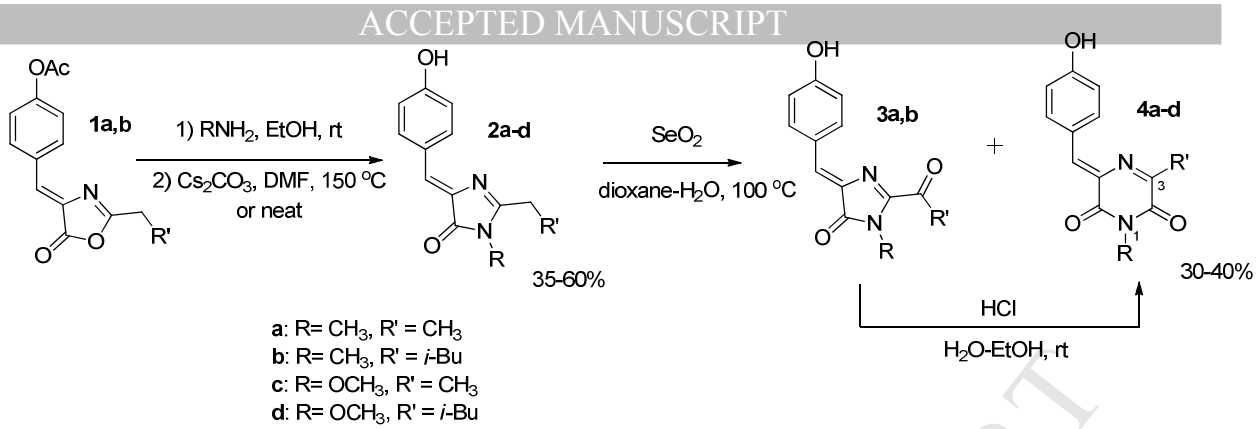


Fig 2. Ring-expanding rearrangement of the red fluorescent proteins chromophores with proposed mechanism. Alk = $(CH_2)_2SMe$: AsFP595 chromoprotein; Alk = $(CH_2)_4NH_2$: ZFP538 fluorescent protein.

The mechanism of this transformation presumably includes protonation of imidazole nitrogen, nucleophilic ring opening upon addition of a water molecule and finally, recyclization at the keto-group .

We found the reaction to proceed easily in polar protonic solvents at acid concentrations in the broad range 0.1–5 M. The maximal yields were achieved using aqueous hydrochloric acid diluted with ethanol to 0.5–1M. The conversion is complete upon several hours at room temperature.

The reaction was found to be non-sensitive to the nature of aliphatic substituent in acyl group (R') and little sensitive to the nature of a substituent at nitrogen atom (R). This allowed us to synthesize a series of flutimide analogs with different substituents at positions 1 and 3 (Scheme 1).



Scheme 1. Synthesis of flutimide analogs **4a-d** using the ring-expanding rearrangement.

The 2-acylimidazolones **3** were obtained by oxidation of the corresponding 2-alkylimidazolones **2** with selenium dioxide, earlier described by us.[7a] This synthetic step was found to be incompatible with unprotected *N*-hydroxyimidazolones. Also, the acidic conditions of 2,6-diketopiperazine ring formation excluded using acid-labile protecting groups at *N*-hydroxy group. Therefore, we used *N*-methoxyimidazolones **2c,d** and *N*-methylated imidazolones **2a,b**.

We found that addition of water to the reaction mixture during the oxidation with SeO_2 in some cases led to direct formation of the rearrangement product. This effect is minute for *N*-methylated analogs **2a,b** whereas in case of *N*-methoxyimidazolones **2c,d** a complete conversion was achieved. The overall yields at the two stages (oxidation-recyclization) were 30–40% both for consecutive and one-pot methods.

Since NMR and other spectral analyses of the rearrangement products provided only indirect evidence of 2,6-diketopiperazine core formation, we performed an X-ray crystallographic study of compound **4d** (Fig 3)[8]

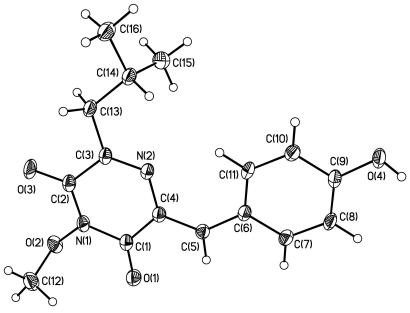
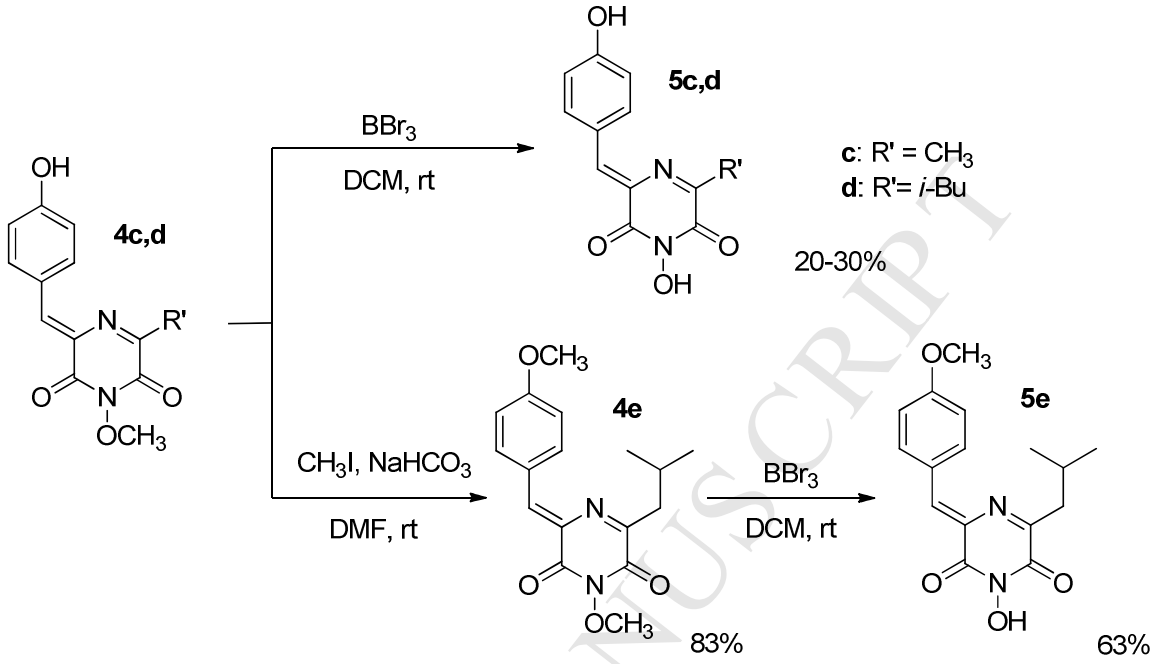


Fig 3. General view of compound **4d** in representation of atoms via thermal ellipsoids at 50% probability level.

Deprotection of the *O*-methylated *N*-hydroxy functionality was completed by using boron tribromide (Scheme 2). We found that demethylation of *N*-hydroxy group occurs faster than

demethylation of phenolic oxygen, this allowed us to selectively deprotect the *N*-OMe moiety in dimethoxy derivative **4e** to obtain compound **5e**.



Scheme 2. Synthesis of flutimide analogs.

Evaluation of antiviral activities and toxicities of compounds **4** and **5** was done on MDCK (Madin-Darby canine kidney) cells, infected with Rimantadine-resistant influenza virus (H1N1 subtype). Among the compounds synthesized, **5e** was identical to the compound **1c** from the paper by Singh et al,[5a] which provided reference to the earlier obtained data. Indeed, the *in vivo* antiviral activity of compound **5e** (IC_{50} 3.0 μM) was comparable with the earlier reported transcriptase inhibitory activity of the same compound (0.8 μM). Expectedly, the IC_{50} of **5e** increased upon transition from an enzyme-based assay to the cell-based assay.

Table 1

CC_{50}, IC_{50} and therapeutic indices of compounds **4** and **5**.

Compound	CC_{50}[a](μM)	IC_{50}[b] (μM)	Therapeutic index[c]
4a	511	>>200	-
4b	-	>>200	-
4c	150	62	2.5
4d	33	25	1.3
4e	160	54	2.9
5c	81	17	4.8
5d	14	4.5	3.0
5e	9.9	3.0	3.3

[a] 50% cytotoxic concentration (CC_{50}; μM);

[b] 50% inhibitory concentration (IC_{50}; μM);

[c] 50% therapeutic index (IC_{50}/CC_{50} ratio; non-dimensional).

The compounds with a free *N*-hydroxyl (**5c**-**e**) possessed the highest antiviral activities, which is in agreement with the data by Singh et al (Table 1). However, the *N*-methoxy derivatives **4c**-**e** appeared to have noticeable activities, whereas the MOM-protected analogs described in[5a] (IC_{50} 25–62μM) did not inhibit the viral transcriptase(IC_{50} >200 μM). The *N*-methylated derivatives **4a,b** were found to be the least active. The compounds bearing isobutyl substituent at the position 3 of diketopiperazine core (**4d,e** and **5d,e**) were more active than their 3-methylated homologs.

Interestingly, the flutimide analogs tested possessed similar therapeutic indices (IC_{50}/CC_{50} ratio), which indicates a link between their antiviral activity and toxicity.

3. Conclusion

In the present report a novel synthetic approach to substituted 2,6-diketopiperazines, including analogs of antiviral candidate flutimide is described. The applicability of this approach for synthesis of diverse 2,6-diketopiperazines is supported by availability of a broad range of precursor imidazolones **2**.[9]

4. Experimental section

CAUTION: Selenium dioxide is extremely poisonous and proper care should be taken when working with it.

4.1. General

Commercially available reagents were used without additional purification. For column chromatography, E. Merck Kieselgel 60 was used. NMR spectra were recorded on a 700-MHz Bruker Avance III NMR at 293 K. Chemical shifts are reported relative to residue peaks of $CDCl_3$ (7.27 ppm for ^1H and 77.0 ppm for ^{13}C) or DMSO-d6 (2.51 ppm for ^1H and 39.5 ppm for ^{13}C). Melting points were measured on a SMP 30 apparatus. IR spectra were obtained on Nicolet iS10 FT-IR spectrometer (Thermo Fisher Scientific Inc. USA). Elemental analyses were performed at the microanalysis facility of Nesmeyanov Intsitute of Organoelement Compounds (Moscow, Russia). High-resolution mass spectra were obtained on an Agilent 6224 TOF LC/MS System (Agilent Technologies, Santa Clara, CA, USA) equipped with a dual-nebulizer ESI source. Data acquisition and analysis was performed by the MassHunter Workstation software (Agilent Technologies, Santa Clara, CA, USA).

Purity of compounds (**4a-e**, **5c-e**) used in biological test was analyzed by HPLC using Waters Breeze Series liquid chromatograph with Diode-array detector 2998 (detection in range 210 nm to 800 nm). The Ascentis (Supelco, USA) RP-Amide column (150mm×4.6 mm, 3 µm particle diameter) was operated at 35 ^oC, flow rate 1.0 mL/min, mobile phase was mixture of acetonitrile and water (1:1 v/v).

4.2. (Z)-4-(4-Acetoxybenzylidene)-oxazol-5(4*H*)-ones

*4.2.1 (Z)-4-(4-Acetoxybenzylidene)-2-methylloxazol-5(4H)-one (**1a**)* was synthesized as reported previously.[7a]

*4.2.2 (Z)-4-(4-Acetoxybenzylidene)-2-isopentyloxazol-5(4H)-one (**1b**).* To the solution of 2-(4-methylpentanamido)acetic acid (38.0 g, 0.22 mol) in dry THF (100 mL), DCC (45 g, 0.22 mol) in 100 mL of THF was added. The mixture was stirred for 24h, filtered and evaporated. 4-acetoxy-benzaldehyde (32.8 g, 200 mmol), NaOAc (16.4 g, 200 mmol) and acetic anhydride (150 mL) was added. The mixture was stirred at 35 ^oC for 3 days, the acetic anhydride was removed in vacuo and the product was purified by chromatography (EtOAc:hexane 1:1; R_f = 0.35). The title compound was obtained as an yellowish solid (31.1 g, 52%); mp 106–108 ^oC; ^1H NMR ($CDCl_3$) δ 8.13 (d, *J* 8.8 Hz, 2H), 7.20 (d, *J* 8.8 Hz, 2H), 7.11 (s, 1H), 2.67 (t, *J* 7.5 Hz, 2H), 2.33 (s, 3H), 1.75–1.69 (m, 3H), 0.99 (d, *J* 6.2 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 21.1 (CH), 22.2 ($2CH_3$), 27.5 (CH_2), 27.7 (CH_2), 33.9 (CH_3), 122.1 (2CH), 129.9 (CH), 130.9, 132.6, 133.5 (2CH), 152. 7, 167.8, 168.9, 169.6; HRMS (ESI) calcd for $C_{17}H_{19}NO_4$ ($[M+H]^+$) 302.1387, found 302.1379.

4.3. (Z)-4-(4-Hydroxybenzylidene)-1H-imidazol-5(4*H*)-ones

*4.3.1 (Z)-2-Ethyl-4-(4-hydroxybenzylidene)-1-methyl-1H-imidazol-5(4H)-one (**2a**)* was synthesized as reported previously.[7a]

*4.3.1 (Z)-2-Isopentyl-4-(4-hydroxybenzylidene)-1-methyl-1H-imidazol-5(4H)-one (**2b**).* In a solution of 3.0g (10 mmol) of **1b** in 50 mL of ethanol the solution of methylamine (wt 40%, 2.0 g, 26 mmol) was added. The mixture was stirred for 20 min, acidified, filtered and evaporated. The crude product was dissolved in 20 mL of DMF and 330mg (1 mmol) of Cs_2CO_3 was added. The mixture was refluxed for 5–10 min (observed by TLC – EtOH:$CHCl_3$ 1:10), evaporated, dissolved in water (50 mL) and acidified by HCl to pH = 3. The mixture was extracted by EtOAc (3 * 50 mL), washed by water (1 * 50 mL), brine, dried over anhydrous Na_2SO_4. The solvent was evaporated and the product was purified by column chromatography (EtOH:$CHCl_3$ 1:15; R_f = 0.45). Yellowish solid (1.62 g, 60%); mp 175–177 ^oC; ^1H NMR (DMSO) δ 10.08 (s, 1H), 8.08 (d, *J* 8.9 Hz, 2H), 6.88 (s, 1H), 6.84 (d, *J* 8.9 Hz, 2H), 3.08 (s, 3H), 2.61 (t, *J* 7.8 Hz, 2H), 1.70–1.65 (m, 1H), 1.62 (q, *J* 8.0 Hz, 2H), 0.95 (d, *J* 6.7 Hz, 6H); ^{13}C NMR (DMSO) δ 22.2 ($2CH_3$), 25.9 (CH_2), 26.0 (CH), 27.3 (CH_2), 33.6 (CH_3), 115.7 (2CH), 125.4, 125.5 (CH), 134.1 (2CH), 136.2, 159.5, 164.9, 170.0; IR (neat) 3130 (w), 2950 (m), 2360 (m), 1670 (s), 1636 (s), 1580 (m), 1460 (m), 1235 (ws), 1160 (m), 820 (m) cm^{-1}; Anal. Calcd for $C_{16}H_{20}N_2O_2$: C, 70.56; H, 7.40; N, 10.29. Found: C, 70.64; H, 7.63; N, 10.25. HRMS (ESI) calcd for $C_{16}H_{20}N_2O_2$ ($[M+H]^+$) 273.1598 found 273.1605.

*4.3.3 (Z)-2-Ethyl-4-(4-hydroxybenzylidene)-1-methoxy-1H-imidazol-5(4H)-one (**2c**).* In a suspension of **1a (**3.5g (13 mmol)) in 50 mL of ethanol the solution of O-methylhydroxylamine was added (the solution was prepared by dilution of amine hydrochloride (2.6g (31 mmol) and 1.1g (28 mmol) of NaOH in 50 mL of ethanol and 5 mL of water). The mixture was stirred for 20 min, acidified, filtered and evaporated. The crude product was dissolved in 20 mL of DMF and 330mg (1 mmol) of Cs_2CO_3 was added. The mixture was refluxed for 5–10 min (observed by TLC – EtOH:$CHCl_3$ 1:10 ; R_f = 0.65), evaporated, dissolved in water (50 mL) and acidified by HCl to pH = 3. The mixture was extracted by EtOAc (3 * 50 mL), washed by water, brine, dried over anhydrous Na_2SO_4. The solvent was evaporated and the product was purified by column chromatography (EtOH:$CHCl_3$ 1:15; R_f = 0.50). The title compound was obtained as an yellowish solid (1.12 g, 35%); mp 180–183 ^oC; ^1H NMR (DMSO) δ 10.19 (s, 1H), 8.10 (d, *J* 8.8 Hz, 2H), 6.97 (s, 1H), 6.84 (d, *J* 8.8 Hz, 2H), 3.94 (s, 3H), 2.69 (q, *J* 7.3 Hz, 2H), 1.27 (t, *J* 7.3 Hz, 3H); ^{13}C NMR (DMSO) δ 9.1 (CH_3), 20.0 (CH_2), 65.0 (CH_3), 115.8 (2CH), 124.9, 127.7 (CH), 132.2, 134.7 (2CH), 160.1, 161.7, 164.3; IR (neat) 3130 (w), 2980 (m), 2360 (m), 1580 (m), 1556 (s), 1386 (m), 1290 (s), 1189 (ws), 1168 (s), 1135 (s), 1106 (s), 1082 (s), 953 (m), 881

(m) cm^{-1}; Anal. Calcd for $C_{13}H_{14}N_2O_3$: C, 63.40; H, 5.73; N, 11.38. Found: C, 63.44; H, 5.87; N, 11.38; HRMS (ESI) calcd for $C_{13}H_{14}N_2O_3$ ($[M+H]^+$) 247.1077, found 247.1099.

*4.3.4 (Z)-2-Isopentyl-4-(4-hydroxybenzylidene)-1-methoxy-1H-imidazol-5(4H)-on (**2d**).* In a solution of **1b** (30.1g (100 mmol)) in 200 mL of ethanol the solution of O-methylhydroxylamine was added (the solution was prepared by dilution of amine hydrochloride [10.0 g (120 mmol) and 4.4 g (110 mmol) of NaOH in 150 mL of ethanol and 20 mL of water]. The mixture was stirred for 30 min, acidified, filtered and evaporated. The mixture was dissolved in 750 mL of EtOAc, washed by water (1 * 150 mL), brine, dried over anhydrous Na_2SO_4 and evaporated. The crude product was heated at 180 ^oC for 5 minutes, cooled to room temperature and dissolved in 500 mL of ethanol. The mixture was stirred with aqueous ammonia (25%, 15 mL) for 10 minutes. The solvent was evaporated (temperature below 40 ^oC) and the mixture was dissolved in 750 mL of EtOAc, acidified, washed by water (2 * 150 mL), brine and dried over anhydrous Na_2SO_4. The solvent was evaporated and the product was purified by column chromatography (EtOAc:hexane 1:1; R_f = 0.35). Yellowish solid (10.7 g, 37%); mp 120–122 ^oC; ^1H NMR ($CDCl_3$) δ 8.05 (d, *J* 8.8 Hz, 2H), 7.12 (s, 1H), 7.00–6.90 (brs, 1H), 6.91 (d, *J* 8.8 Hz, 2H), 4.02 (s, 3H), 2.65 (t, *J* 7.9 Hz, 2H), 1.75–1.70 (m, 3H), 0.99 (d, *J* 6.3 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 22.2 ($2CH_3$), 25.4, 27.9, 34.4, 65.3 (CH_3), 116.1 (2CH), 126.4, 129.8 (CH), 132.8, 134.9 (2CH), 158.8, 161.1, 165.8; IR (neat) 3145 (w), 2950 (m), 2361 (m), 1680 (s), 1638 (s), 1580 (m), 1510 (s), 1460 (m), 1230 (m), 1140 (m), 1103 (s), 1065 (m), 993 (s), 946 (s), 854 (m) cm^{-1}; Anal. Calcd for $C_{16}H_{20}N_2O_3$: C, 66.65; H, 6.99; N, 9.72. Found: C, 66.69; H, 6.84; N, 9.64; HRMS (ESI) calcd for $C_{16}H_{20}N_2O_3$ ($[M+H]^+$) 289.1547 found 289.1556.

4.4. General procedure of oxidation

To the solution of **2** (25 mmol) in dioxane (200 mL), the selenium dioxide (5.50 g, 50 mmol) and water (15 mL) was added. The mixture was refluxed for 15 minutes (monitored by TLC (EtOH:$CHCl_3$, alumina oxide)). The solvent was evaporated and the mixture was dissolved in EtOAc (400 mL), washed by water (2 * 50 mL), brine, dried over anhydrous Na_2SO_4. The solvent was evaporated and the products were purified by column chromatography.

*4.4.1 Oxidation of **2a** and **2b***

In these reactions only ketone products **3** were formed:

*(Z)-2-Acetyl-4-(4-hydroxybenzylidene)-1-methyl-1H-imidazol-5(4H)-one (**3a**).* Described previously.[7a]

*(Z)-4-(4-Hydroxybenzylidene)-1-methyl-2-(3-methylbutanoyl)-1H-imidazol-5(4H)-one (**3b**).* Red solid (458 mg, 64%); mp 202–204 ^oC; (EtOH:$CHCl_3$ 1:10; R_f = 0.40); ^1H NMR (DMSO) δ 10.60–10.50 (brs, 1H), 8.20 (d, *J* 8.9 Hz, 2H), 7.37 (s, 1H), 6.91 (d, *J* 8.9 Hz, 2H), 3.29 (s, 3H), 3.00 (d, *J* 6.7 Hz, 2H), 2.25–2.10 (m, 1H), 0.98 (d, *J* 6.7 Hz, 6H); ^{13}C NMR

(DMSO) δ 22.4 ($2CH_3$), 24.3, 28.3, 46.5 (CH_3), 116.3 (2CH), 124.9, 134.2 (CH), 135.3, 135.7 (2CH), 153.5, 161.5, 169.6, 194.9 (CO); HRMS (ESI) calcd for $C_{16}H_{18}N_2O_3$ ($[M+H]^+$) 287.1390 found 287.1408.

*4.4.2 Oxidation of **2c** and **2d***

In these case formation of 3H-pyrazine-2,6-diones **4** were observed as major process:

*(Z)-3-(4-Hydroxybenzylidene)-1-methoxy-5-methylpyrazine-2,6(1H,3H)-dione **(4c)**.* Orange solid (2,02 g, 31%); mp ~ 210 ^oC with decomposition; (EtOH:$CHCl_3$ 1:10; R_f = 0.35); ^1H NMR (DMSO) δ 10.51 (s, 1H), 8.20 (d, *J* 8.8 Hz, 2H), 7.63 (s, 1H), 6.88 (d, *J* 8.8 Hz, 2H), 3.84 (s, 3H), 2.37 (s, 3H); ^{13}C NMR (DMSO) δ 20.7 (CH3), 6.3 (CH3), 116.1 (2CH), 125.1, 130.7, 136.5 (2CH), 140.4 (CH), 154.4, 154.5, 159.7, 161.5; IR (neat) 3330 (w), 1702 (ws), 1652 (ws), 1597 (ws), 1542 (ws), 1510 (m), 1370 (m), 1170 (m), 1145 (m), 1046 (s), 845 (m) cm^{-1}; Anal. Calcd for $C_{13}H_{12}N_2O_4$*0.33H_2O: C, 58.64; H, 4.80; N, 10.52. Found: C, 58.68; H, 4.91; N, 10.52; HRMS (ESI) calcd for $C_{13}H_{12}N_2O_4$ ($[M+H]^+$) 261.0870 found 261.0865.

*(Z)-3-(4-Hydroxybenzylidene)-5-isobutyl-1-methoxypyrazine-2,6(1H,3H)-dione **(4d)**.* Orange solid (2.44 g, 33%); mp 205–208 ^oC; (EtOH:$CHCl_3$ 1:15; R_f = 0.45); ^1H NMR ($CDCl_3$) δ 8.20 (d, *J* 8.7 Hz, 2H), 7.82 (s, 1H), 6.95 (d, *J* 8.7 Hz, 2H), 5.90–5.85 (brs, 1H), 4.02 (s, 3H), 2.76 (d, *J* 7.2 Hz, 2H), 2.35–2.15 (m, 1H), 1.06 (d, *J* 6.5 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 22.6 ($2CH_3$), 26.5 (CH), 41.9 (CH_2), 64.2 (CH_3), 116.2 (2CH), 126.9, 131.1, 136.9 (2CH), 142.9 (CH), 154.6, 158.9, 159.7, 160.3; IR (neat) 3260 (w), 2960 (m), 1702 (ws), 1658 (ws), 1603 (ws), 1538 (ws), 1505 (m), 1360 (m), 1250 (ws), 1210 (m), 1170 (m), 860 (m) cm^{-1}; Anal. Calcd for $C_{16}H_{18}N_2O_4$: C, 63.56; H, 6.00; N, 9.27. Found: C, 63.54; H, 5.94; N, 9.36; HRMS (ESI) calcd for $C_{16}H_{18}N_2NaO_4$ ($[M+Na]^+$) 325.1164 found 325.1153.

4.5. Recyclization in acidic conditions

The solution of **3a-b** (1 mmol) in ethanol (20 mL) was cooled to 0 ^oC, and the concentrate solution of HCl (36%, 3.5 mL) was added. The mixture was heated to room temperature and stirred overnight (monitored by TLC (EtOH:$CHCl_3$, alumina oxide)). The mixture was dissolved in EtOAc (100 mL) and water (50 mL) and excess of $NaHCO_3$ was added. The organic layer was separated, washed by water (2 * 30 mL), brine and dried over anhydrous Na_2SO_4. The solvent was evaporated and the product was purified by column chromatography.

*4.5.1 (Z)-3-(4-Hydroxybenzylidene)-1,5-dimethylpyrazine-2,6(1H,3H)-dione **(4a)**.* Orange solid (160 mg, 66%); mp ~200 ^oC with decomposition; (EtOH:$CHCl_3$ 1:10; R_f = 0.35); ^1H NMR (DMSO) δ 10.43 (s, 1H), 8.19 (d, *J* 8.8 Hz, 2H), 7.62 (s, 1H), 6.88 (d, *J* 8.8 Hz, 2H), 3.15 (s, 3H), 2.36 (s, 3H); ^{13}C NMR (DMSO) δ 21.4 (CH_3), 40.9 (CH_3), 116.5 (2CH), 132.5, 136.7 (2CH), 140.3 (CH), 154.7, 158.1, 161.6, 163.2, 163.8; HRMS (ESI) calcd for $C_{13}H_{12}N_2O_3$ ($[M+H]^+$) 245.0921 found 245.0934.

*4.5.2 (Z)-3-(4-Hydroxybenzylidene)-5-isobutyl-1-methylpyrazine-2,6(1H,3H)-dione (**4b**).* Orange solid (163 mg, 57%); mp ~280 ^{o}C with decomposition; (EtOH:$CHCl_3$ 1:10; R_f = 0.50); ^{1}H NMR (DMSO) δ 10.44 (s, 1H), 8.19 (d, *J* 8.8 Hz, 2H), 7.62 (s, 1H), 6.87 (d, *J* 8.8 Hz, 2H), 2.61 (d, *J* 7.0 Hz, 2H), 2.25–2.20 (m, 1H), 0.98 (d, *J* 6.7 Hz, 6H); ^{13}C NMR (DMSO) δ 22.4 ($2CH_3$), 25.6 (CH_2), 25.7 (CH_2), 41.6 (CH_3), 115.9 (2CH), 125.2, 130.1, 136.1 (2CH), 140.1 (CH), 155.9, 157.4, 161.1, 162.5; HRMS (ESI) calcd for $C_{13}H_{12}N_2O_3$ ($[M+H]^+$) 287.1390 found 287.1377.

4.6. Demethylation by boron tribromide

Compound **4** (0.5 mmol) were dissolved in dry dichloromethane (3 mL), cooled to 0 ^{o}C and the solution of boron tribromide in dichloromethane (1M, 1 mL) was added. The mixture was stayed at room temperature for 5 minutes and after that 50 mL of dichloromethane and 20 mL of water was added. The $NaHCO_3$ was added to pH = 5. The organic layer was washed by water (2 * 30 mL), brine and dried over Na_2SO_4. The solvent was evaporated (at the temperature below 40 ^{o}C) and the product was purified by column chromatography.

*4.6.1 (Z)-1-Hydroxy-3-(4-hydroxybenzylidene)-5-methylpyrazine-2,6(1H,3H)-dione (**5c**).* Orange solid (26 mg, 21%); mp ~210 ^{o}C with decomposition; (EtOH:$CHCl_3$ 1:10; R_f = 0.35); ^{1}H NMR (DMSO) δ 10.80 (s, 1H), 10.49 (s, 1H), 8.20 (d, *J* 8.8 Hz, 2H), 7.63 (s, 1H), 6.89 (d, *J* 8.8 Hz, 2H), 2.37 (s, 3H); ^{13}C NMR (DMSO) δ 20.8 (CH_3), 116.1 (2CH), 125.1, 130.7, 136.5 (2CH), 140.4 (CH), 154.4, 154.9, 160.5, 161.4; IR (neat) 3200 (w), 2940 (m), 2360 (m), 1640 (m), 1543 (s), 1460 (m), 1410 (ws), 1320 (m), 1260 (ws), 1050 (m), 970 (m), 840 (m) cm^{-1}; HRMS (ESI) calcd for $C_{12}H_{10}N_2O_4$ ($[M+H]^+$) 247.0713 found 247.0719.

*4.6.2 (Z)-1-Hydroxy-3-(4-hydroxybenzylidene)-5-isobutylpyrazine-2,6(1H,3H)-dione (**5d**).* Isolated as monohydrate. Orange solid (41 mg, 28%); mp ~220 ^{o}C with decomposition; (EtOH:$CHCl_3$ 1:10; R_f = 0.40); ^{1}H NMR (DMSO) δ 10.80–10.70 (brs, 1H), 10.60–10.55 (brs, 1H), 8.21 (d, *J* 8.7 Hz, 2H), 7.65 (s, 1H), 6.88 (d, *J* 8.7 Hz, 2H), 2.62 (d, *J* 6.9 Hz, 2H), 2.22–2.12 (m, 1H), 0.99 (d, *J* 6.5 Hz, 6H); ^{13}C NMR (DMSO) δ 22.3 ($2CH_3$), 25.9 (CH), 39.3 (CH_2), 116.0 (2CH), 125.2, 130.6, 136.4 (2CH), 140.9 (CH), 154.9, 156.1, 160.3, 161.4; IR (neat) 3420 (w), 2950 (m), 2360 (m), 1684 (s), 1580 (ws), 1570 (ws), 1434 (s), 1317 (s), 1210 (m), 1190 (ws), 1120 (m), 990 (m), 858 (s), 760 (m) cm^{-1}; Anal. Calcd for $C_{15}H_{16}N_2O_4*H_2O$: C, 58.82; H, 5.92; N, 9.15. Found: C, 58.93; H, 5.81; N, 9.02; HRMS (ESI) calcd for $C_{15}H_{16}N_2O_4$ ($[M+H]^+$) 289.1183 found 289.1187.

4.7. (Z)-1-Hydroxy-5-isobutyl-3-(4-methoxybenzylidene)pyrazine-2,6(1*H*,3*H*)-dione (5e)

*4.7.1 (Z)-5-Isobutyl-1-methoxy-3-(4-methoxybenzylidene)pyrazine-2,6(1H,3H)-dione (**4e**).* To the solution of **4d** (302 mg, 1.0 mmol) in dry DMF (30 mL), the methyl iodide (560 mg, 4

mmol) and $NaHCO_3$ (340 mg, 4 mmol) was added. The mixture was stirred for 50 minutes (monitored by TLC (EtOH:$CHCl_3$ 1:20), diluted with EtOAc (150 mL), washed by water (2 * 50 mL), brine and dried over Na_2SO_4. The solvent was evaporated and the product was purified by column chromatography ($CHCl_3$; R_f = 0.30). Yellowish solid (262 mg, 83%); mp 144–146 ^oC; ^1H NMR ($CDCl_3$) δ 8.23 (d, *J* 8.8 Hz, 2H), 7.81 (s, 1H), 6.98 (d, *J* 8.8 Hz, 2H), 4.01 (s, 3H), 3.90 (s, 3H), 2.75 (d, *J* 7.1 Hz, 2H), 2.33–2.25 (m, 1H), 1.06 (d, *J* 6.6 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 22.6 ($2CH_3$), 26.5 (CH), 41.9 (CH_2), 55.5 (CH_3), 64.1 (CH_3), 114.6 (2CH), 126.8, 131.2, 136.5 (2CH), 142.8 (CH), 154.6, 156.8, 160.1, 163.0; IR (neat) 2940 (m), 2360 (m), 1715 (s), 1700 (ws), 1673 (s), 1590 (m), 1555 (s), 1440 (m), 1350 (m), 1160 (ws), 1030 (ws), 826 (s), 740 (m) cm^{-1}; Anal. Calcd for $C_{17}H_{20}N_2O_4$: C, 63.54; H, 6.37; N, 8.86. Found: C, 63.73; H, 6.14; N, 8.66; HRMS (ESI) calcd for $C_{17}H_{20}N_2O_4$ ($[M+H]^+$) 317.1496 found 317.1499.

*4.7.2 (Z)-1-Hydroxy-5-isobutyl-3-(4-methoxybenzylidene)pyrazine-2,6(1H,3H)-dione (**5e**).*

A solution of (**4e**) (160 mg, 0.5 mmol) in dry dichloromethane (3 mL) was cooled to 0 ^oC and the solution of boron tribromide in dichloromethane (1M, 0.7 mL) was added. The mixture was stayed at room temperature for 5 minutes and after that 50 mL of dichloromethane and 20 mL of water was added. The $NaHCO_3$ was added to pH = 5. The organic layer was washed by water (2 * 30 mL), brine and dried over Na_2SO_4. The solvent was evaporated (at the temperature below 40 ^oC) and the product was purified by column chromatography ($CHCl_3$:EtOH 10:1; R_f = 0.40). Orange solid (95 mg, 63%); mp 157–159 ^oC (lit 152–156 ^oC[5a]); ^1H NMR ($CDCl_3$) δ 8.27 (d, *J* 7.6 Hz, 2H), 7.86 (s, 1H), 6.99 (d, *J* 7.6 Hz, 2H), 3.91 (s, 3H), 2.76 (d, *J* 3.3 Hz, 2H), 2.35–2.22 (m, 1H), 1.05 (d, *J* 6.1 Hz, 6H); IR (neat) 3080 (w), 2960 (wm), 2360 (m), 1703 (s), 1647 (s), 1603 (s), 1560 (m), 1190 (ws) 1120 (m), 1010 (m), 985 (s), 830 (m) cm^{-1}; HRMS (ESI) calcd for $C_{16}H_{18}N_2O_4$ ($[M+Na]^+$) 325.1159 found 325.1150.

4.8 Cytotoxity and antiviral activity

Cytotoxic effects of the compounds in the MDCK (Madin-Darby canine kidney) cell culture were visually assessed after 48–72 hours of incubation with the compounds investigated. Then the cell monolayer was washed and 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-*2H*-tetrazolium-5-carboxanilide sodium salt (XTT) was added at 1 mg/mL. After 4 hours of incubation the results were counted in an automatic colorimeter at wavelength of 450 nm and a 50% cytotoxic dose was calculated.

Studies of the antiviral activity of flutimide analogs and oseltamivir carboxylate at 1 mkg/mL as positive control were performed on 96-well plates with a monolayer of MDCK cells inoculated by influenza virus A/IIV-Moscow/01/2009 (H1N1) pdm09 (full analogue of prototype A/California/07/2009 (H1N1) pdm09 resistant for rimantadine).[10] Simultaneously with the infection of a monolayer the tested compounds were added at various concentrations. Panels

were incubated for 24 hours at 37 °C and then quenched by fixing the cells with 80% acetone in a phosphate buffer. An enzyme immunoassay technique was performed as described previously[11] to assess concentration of viral proteins.

Acknowledgements

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Supplementary data

HPLC and IR data are available. Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.tet.

References and notes

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8. Crystals of **4d** ($C_{16}H_{18}N_2O_4$, M = 302.32) grown from methanol were colourless, monoclinic, space group P 2_1/n. X-ray diffraction data were collected using a "Bruker SMART APEX2" CCD diffractometer (λ(MoKα) = 0.71073Å, graphite monochromator) at 100(2) K: a =5.9730(4), b = 16.6528(11), c = 14.6606(9) Å; β = 94.5571(14)°. Intensities of 7850 reflections were measured and 3828 independent reflections [R_{int} = 0.0445] were used in further refinement. Initially spherical atom refinements were undertaken with SHELXTL PLUS 5.0 [Sheldrick G.M. *Acta. Cryst*. **2008**, A64, 112–122.] using the full-matrix least-squares method. All non-hydrogen atoms were allowed to have an anisotropic thermal motion. The refinement converged to wR_2 = 0.1059 and GOF = 1.005 for all independent reflections (R_1 = 0.0432 was calculated against F for 2693 observed reflections with I > 2σ(I)). Atomic coordinates, bond lengths, angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Center (CCDC) with number 983835.

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Supporting Information

Ring-expanding rearrangement of 2-acyl-5-arylidene-3,5-dihydro-*4H*-imidazol-4-ones in synthesis of flutimide analogs

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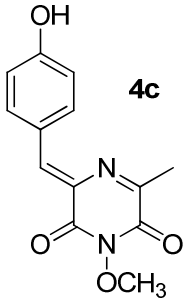
HPLC Profiles of the products **S2**

IR spectra of the products **S8**

HPLC Profiles of the products



4c

SAMPLE INFORMATION

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Injection #:	2	Processing Method	Imp3
Injection Volume:	10.00 ul	Channel Name:	413.5nm
Run Time:	15.0 Minutes	Proc. Chnl. Descr.:	PDA 413.5 nm
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Date Processed:	29-Jun-12 12:58:39 MSD		

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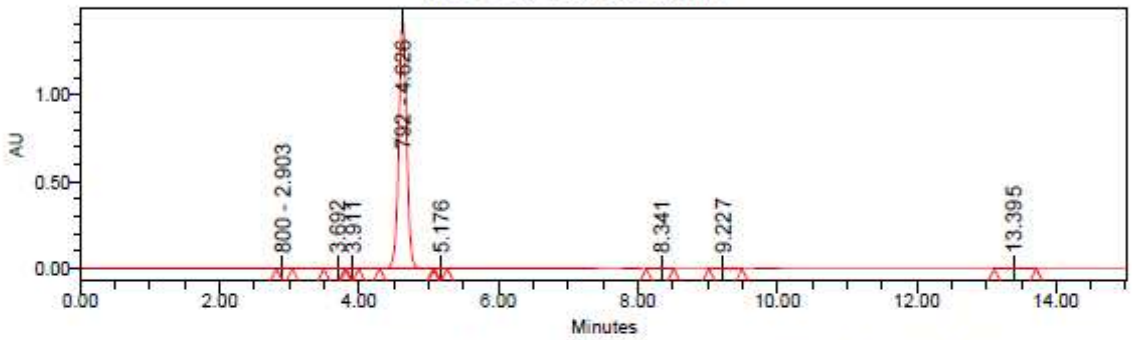

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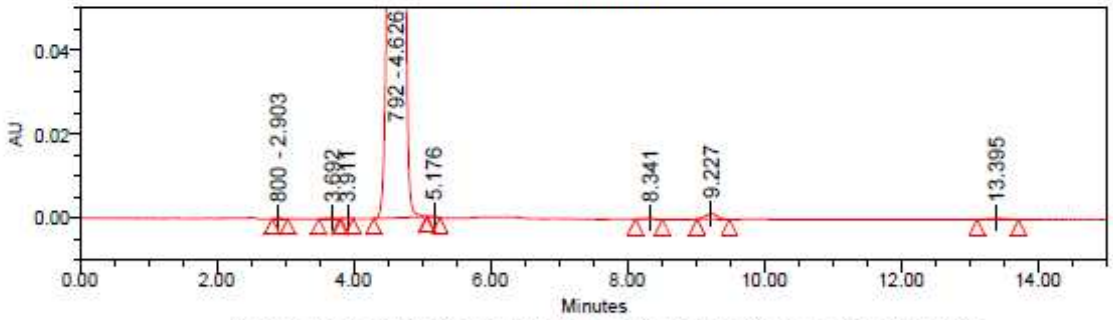

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Peak Results

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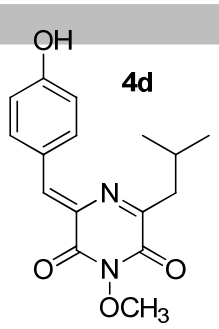
2		3.692	2118	0.02
3		3.911	1106	0.01
4	792	4.626	11184894	99.73
5		5.176	1356	0.01
6		8.341	2934	0.03
7		9.227	15593	0.14
8		13.395	5316	0.05



SAMPLE INFORMATION

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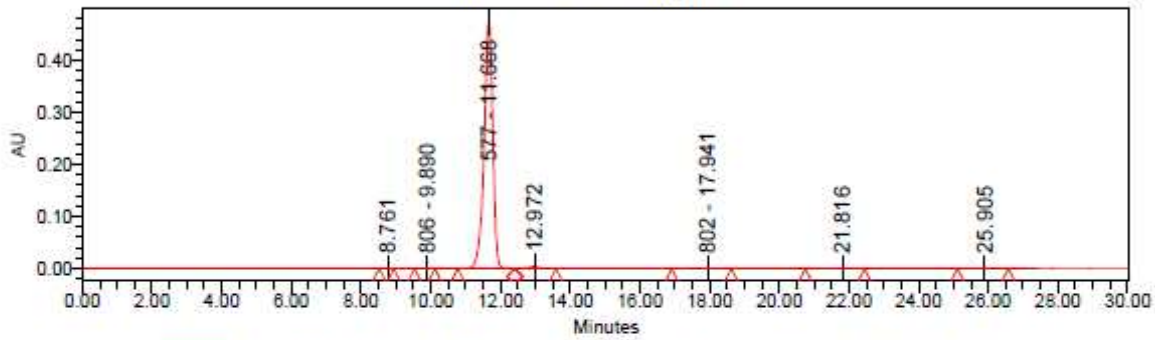
Injection #:	1	Processing Method	Imp3
Injection Volume:	10.00 ul	Channel Name:	410.9nm
Run Time:	30.0 Minutes	Proc. Chnl. Descr.:	PDA 410.9 nm
Date Acquired:	28-Jun-12 22:48:41 MSD		
Date Processed:	29-Jun-12 13:28:39 MSD		

Auto-Scaled Chromatogram



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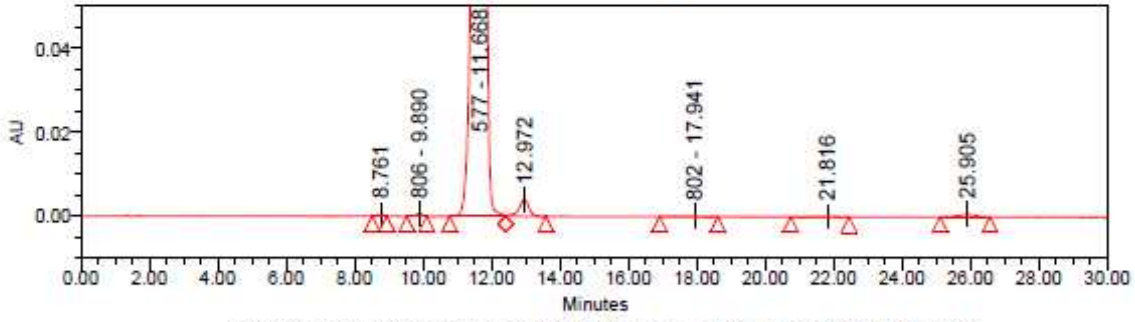

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Peak Results

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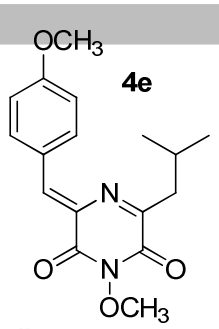
3	577	11.668	8182966	98.48
4		12.972	82689	1.00
5	802	17.941	3334	0.04
6		21.816	4837	0.06
7		25.905	22051	0.27



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Injection Volume:	10.00 ul	Channel Name:	408.1nm
Run Time:	40.0 Minutes	Proc. Chnl. Descr.:	PDA 408.1 nm
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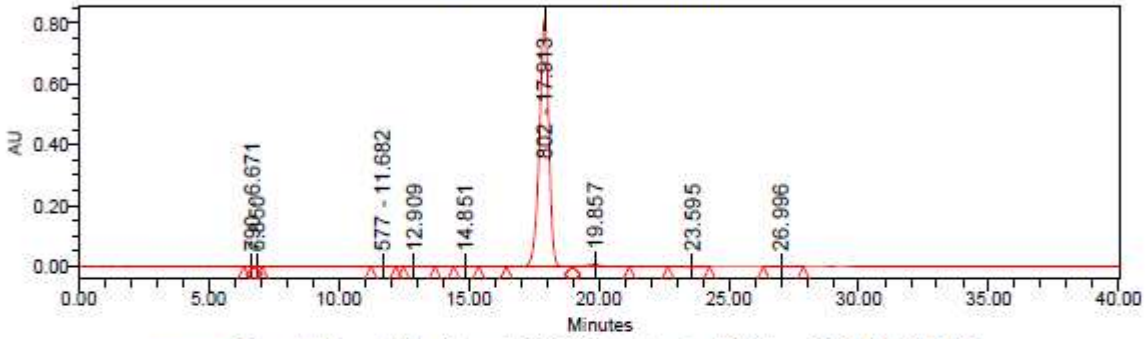
Auto-Scaled Chromatogram



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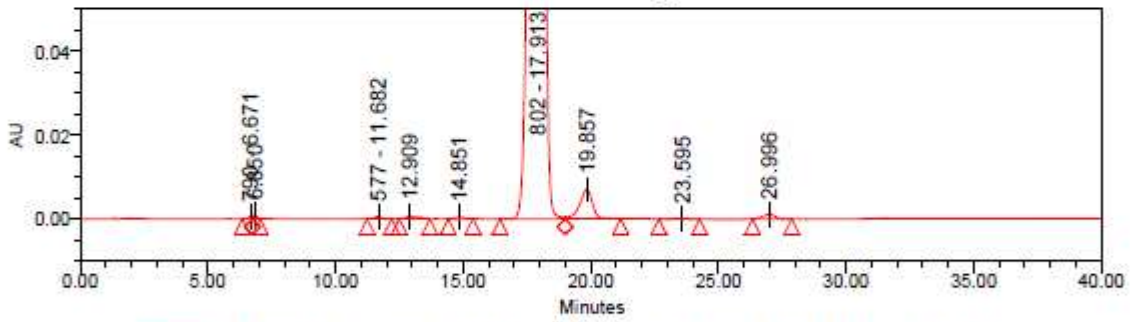

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Peak Results

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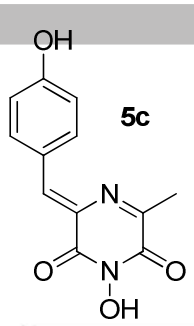
2		6.850	10129	0.05
3	577	11.682	9952	0.05
4		12.909	20750	0.10
5		14.851	9340	0.04
6	802	17.913	20502502	98.35
7		19.857	240643	1.15
8		23.595	5244	0.03

	Name	RT	Area	% Area
9		26.996	40793	0.20



SAMPLE INFORMATION

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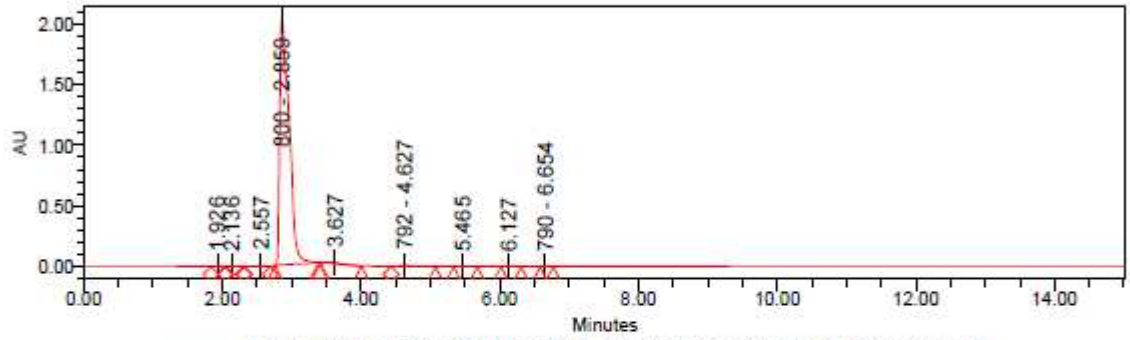
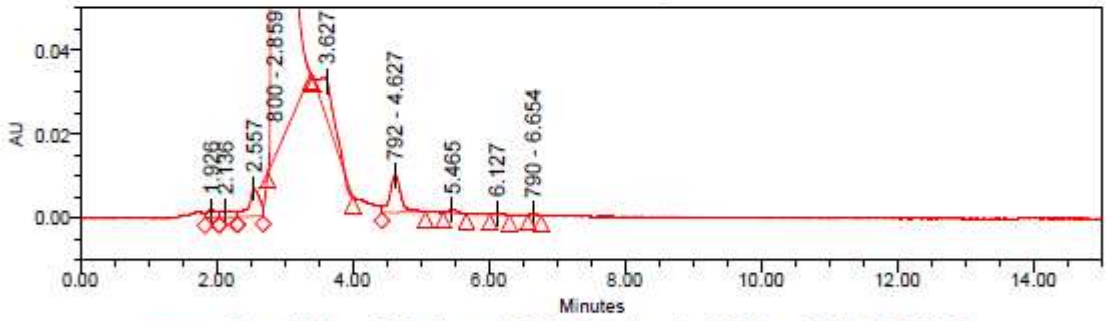
Auto-Scaled Chromatogram



Sample Name 800; Channel 2998; Date Acquired 28-Jun-12 18:18:55 MSD

Auto-Scaled Chromatogram



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Peak Results

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3		2.557	72889	0.38
4	800	2.859	18845382	98.11
5		3.627	138148	0.72
6	792	4.627	101334	0.53
7		5.465	6973	0.04
8		6.127	3173	0.02

	Name	RT	Area	% Area
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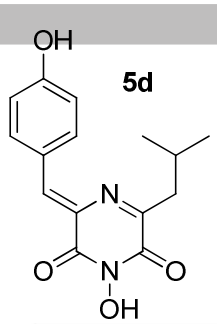

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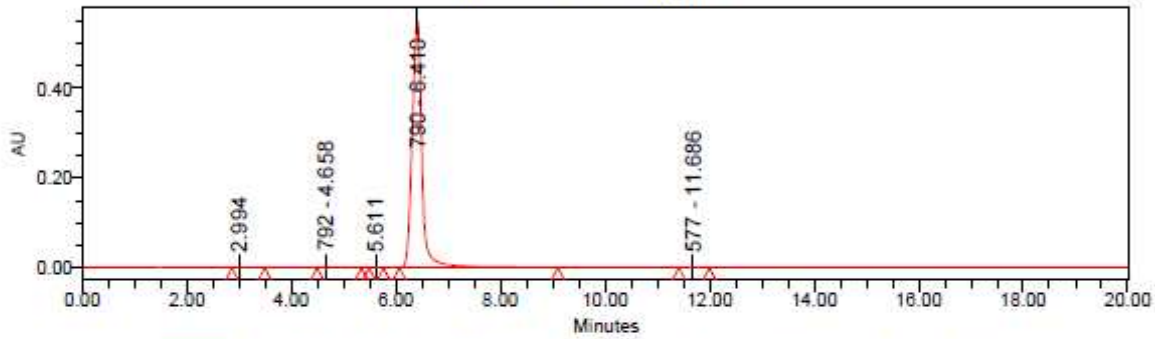
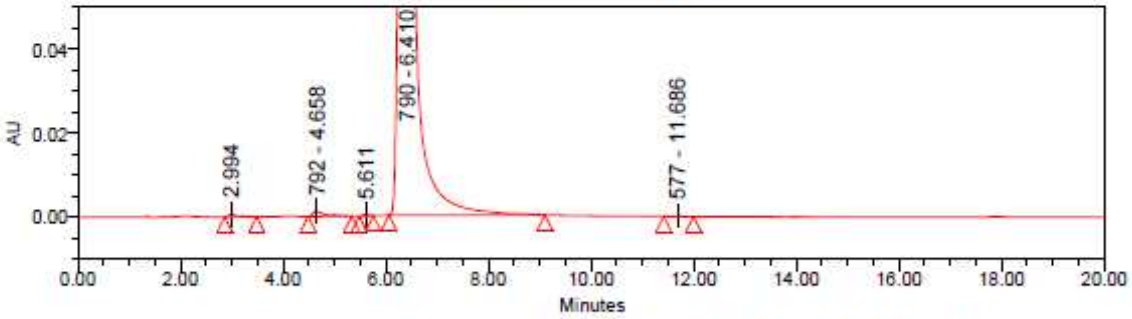
Injection Volume:	10.00 ul	Channel Name:	414.5nm
Run Time:	20.0 Minutes	Proc. Chnl. Descr.:	PDA 414.5 nm
Date Acquired:	28-Jun-12 23:49:27 MSD		
Date Processed:	29-Jun-12 13:16:56 MSD		

Auto-Scaled Chromatogram



Sample Name 790; Channel 2998; Date Acquired 28-Jun-12 23:49:27 MSD

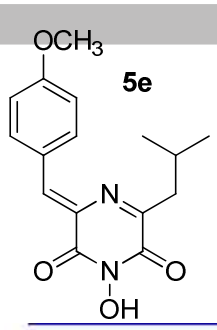
Auto-Scaled Chromatogram



Sample Name 790; Channel 2998; Date Acquired 28-Jun-12 23:49:27 MSD

Peak Results

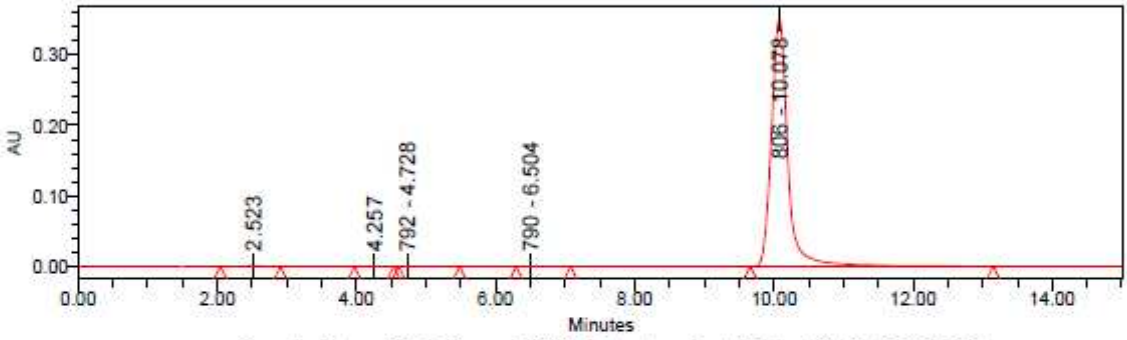
	Name	RT	Area	% Area
1		2.994	8056	0.11
2	792	4.658	21106	0.30
3		5.611	2741	0.04
4	790	6.410	7046110	99.52
5	577	11.686	1883	0.03



SAMPLE INFORMATION

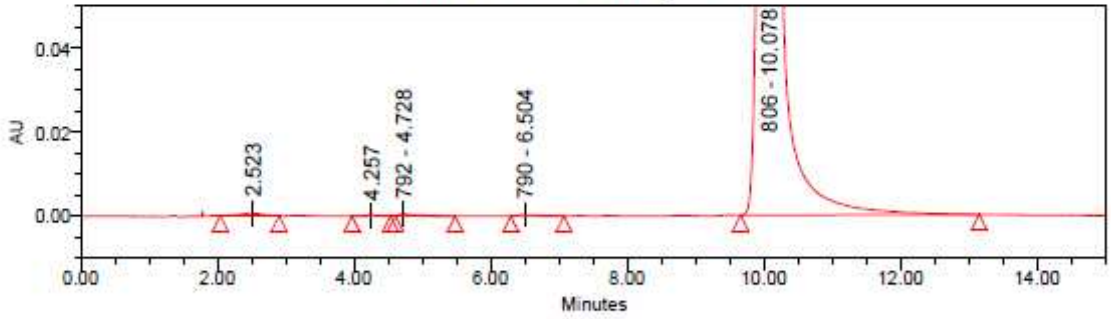
Sample Name:	806	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	260612
Vial:	1:A,6	Acq. Method Set:	50% ACN PDA
Injection #:	2	Processing Method	Imp3
Injection Volume:	10.00 ul	Channel Name:	406.5nm
Run Time:	15.0 Minutes	Proc. Chnl. Descr.:	PDA 406.5 nm
Date Acquired:	28-Jun-12 18:49:39 MSD		
Date Processed:	29-Jun-12 13:25:56 MSD		

Auto-Scaled Chromatogram



Sample Name 806; Channel 2998; Date Acquired 28-Jun-12 18:49:39 MSD

Auto-Scaled Chromatogram



Sample Name 806; Channel 2998; Date Acquired 28-Jun-12 18:49:39 MSD

Peak Results

	Name	RT	Area	% Area
1		2.523	12823	0.22
2		4.257	2168	0.04
3	792	4.728	6785	0.12
4	790	6.504	7100	0.12
5	806	10.078	5733323	99.50

IR spectra of the products