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Design, synthesis and biological activity of flavonoid derivatives as selective agonists for neuromedin U 2 receptor

Ming-Liang Ma [a,b], Ming Li [b], Jiao-Jiao Gou [a], Tian-Yu Ruan [a], Hai-Shan Jin [a], Ling-Hong Zhang [a], Liang-Chun Wu [a], Xiao-Yan Li [a], Ying-He Hu [b], Ke Wen [c,*], Zheng Zhao [a,*]

[a] Key Laboratory of Brain Functional Genomics, Ministry of Education, Shanghai Key Laboratory of Brain Functional Genomics, East China Normal University, Shanghai 200062, China
[b] Shanghai Engineering Research Center of Molecular Therapeutics and New Drug Development, East China Normal University, Shanghai 200062, China
[c] Shanghai Advanced Research Institute, Chinese Academy of Science, Shanghai 201210, China

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ABSTRACT

Central neuromedin U 2 receptor (NMU2R) plays important roles in the regulation of food intake and body weight. Identification of NMU2R agonists may lead to the development of pharmaceutical agents to treat obesity. Based on the structure of rutin, a typical flavonoid and one of the NMU2R agonists we previously identified from an in-house made natural product library, 30 flavonoid derivatives have been synthesized and screened on a cell-based reporter gene assay. A number of compounds were found to be selective and highly potent to NMU2R. For example, the EC_{50} value of compound NRA **4** is very close to that of NMU, the endogenous peptide ligand of NMU2R. Structure–activity relationship analysis revealed that a 3-hydroxyl group in ring C and a 2′-fluoride group in ring B were essential for this class of compounds to be active against NMU2R.

1. Introduction

Neuromedin U (NMU) regulates a variety of physiological activities in mammals, including stimulation of smooth-muscle contraction,[1] blood pressure regulation,[2] ion transport,[3,4] local blood flow control,[5] gastric acid secretion and gastric emptying.[6] In the central nervous system, NMU plays important roles in the regulation of feeding behavior[7] and energy homeostasis.[8] Previous study has identified two G-protein coupled receptors (GPCRs) for NMU, NMU1R and NMU2R.[7] NMU1R is expressed in a wide range of peripheral tissues such as intestine, testis, pancreas, uterus, lung and kidney,[7,9] while NMU2R is expressed exclusively in the central nervous system (CNS), mainly in the paraventricular nucleus (PVN) and arcuate nucleus of the hypothalamus.[9–11] It has been reported that intracerebroventricular (ICV) injection of NMU decreased the food intake and body weight in rodents.[12] Moreover, ICV injection of NMU antiserum in rats attenuated the effects of NMU and increased food intake.[13] Recent studies also showed that the knockout of NMU gene in mice resulted in obesity phenotype and those mice showed the increase of food intake and decrease of energy expenditure.[14] Deng[15] in 2014 reported that *p*-synephrine binding to NMU2R with high efficacy and potency; the EC_{50} of *p*-synephrine is 6.6 μM for the NMU2R, is a highly potent and selective NMU2R agonist. These results suggest that NMU regulates food intake and energy expenditure potentially through the activation of NMU2R in the hypothalamus[16,17] and thus provide a notion that NMU2R is potentially a valuable therapeutic target for the development of new therapeutics for treating obesity.

It is well-established that natural products are excellent structural sources for biological screening. Previously, by screening an in-house made natural product library, we have identified a few natural products which could selectively activate NMU2R in a cell-based assay. Interestingly, in vivo study demonstrated that the expressions of NMU2R and NMU were significantly up-regulated in the hypothalamus of mice at the third or fourth week of the treatment with one of the compounds.[18] Structural analysis showed that these compounds have similar core structure and belong to the family of flavonoid glycosides, which are a group of polyphenolic secondary metabolites present in a wide variety of plants (Fig. 1).[18] However, these compounds generally exhibited low activity toward NMU2R.[18,19] Oriented to the discovery of small molecular NMU2R agonists, we initiated a medicinal chemistry program based on the structural modification of the previously discovered natural product NMU2R agonists, flavonoid glycosides. By maintaining the core structure of the three ring system, 30 compounds have been synthesized and their biological activities in NMU2R cell-based assay have been examined. A number of flavon-3-ol derivatives with strong potency against NMU2R have

* Corresponding authors. Tel.: +86 21 62238005; fax: +86 21 62601953.
E-mail addresses: wenk@sari.ac.cn (K. Wen), zzhao@brain.ecnu.edu.cn (Z. Zhao).

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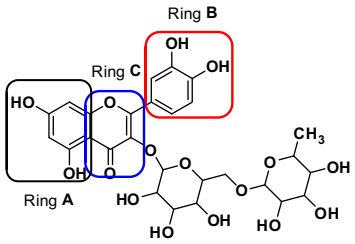


Figure 1. Chemical structure of rutin.

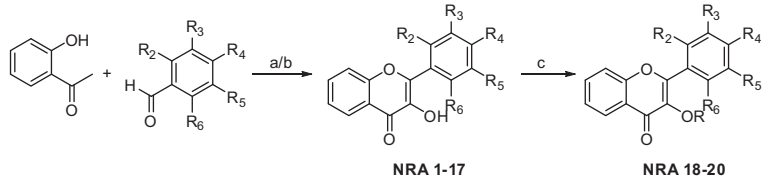
been identified. To our knowledge, this is the first medicinal chemistry program aimed at the discovery of NMU2R agonists.

In this report, we showed the biological activities of flavonol and flavone analogues and discussed the structure–activity relationship (SAR) of this class of compounds.

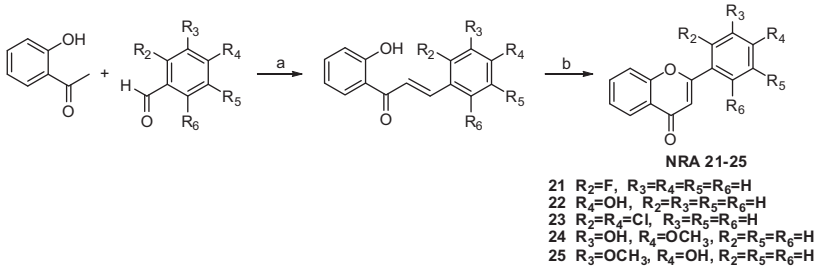
2. Results and discussion

2.1. Chemistry

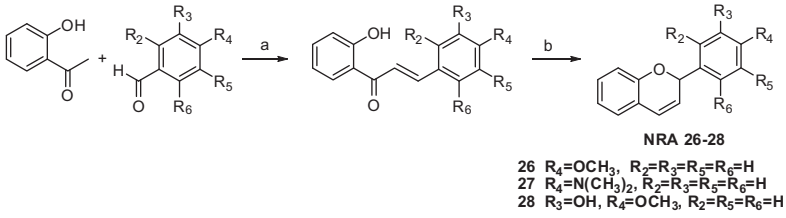
All NRAs (NMU2R Agonists) have been prepared according to literature procedures by employing 2-hydroxyacetophenone and commercially available substituted benzaldehydes as starting materials. Schemes 1–4 illustrate the synthesis leading to the target structures of flavon-3-ols (NRA **1**–**17**), 3-alkoxy-flavones (NRA **18**–**20**), flavones (NRA **21**–**25**) and flav-3-enes (NRA **26**–**30**). Detailed synthetic protocols are presented in the experimental section. NRA **25**–**30** were synthesized as racemics which bear one steric center (Schemes 1–4). Table 1 shows the chemical structures of flavon-3-ols (NRA **1**–**20**) and their EC_{50} values in a cell-based NMU2R assay. Screening test revealed that compounds NRA **21**–**30** showed no biological activities toward NMU2R. Thus, these compounds were not listed in Table 1.



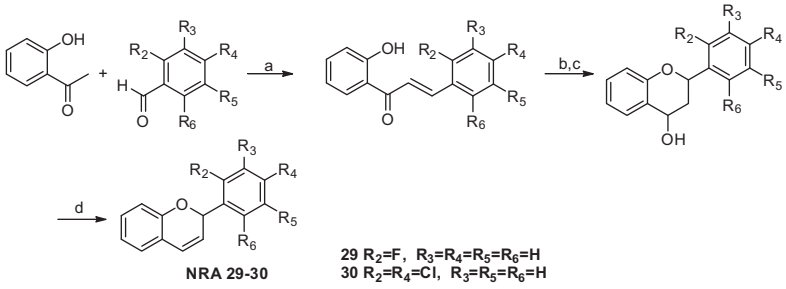
Scheme 1. Synthesis of NRA **1**–**20**. Reagents and conditions: (a) EtOH/1,4-dioxane, 40% w/v KOH, 0 to rt; (b) 1,4-dioxane/EtOH, 5.4% w/v NaOH, 35% H_2O_2, 0 to rt, yield:40–65% (2 steps) (c) RX, K_2CO_3, DMF, rt, yield: 90–95%.



Scheme 2. Synthesis of NRA **21**–**25**. Reagents and conditions: (a) (1) KOH (40%), EtOH, 0 to rt, (2) HCl (6 N); (b) I_2, DMSO, reflux, yield: 40–65% (2 steps).



Scheme 3. Synthesis of NRA **26**–**28**. Reagents and conditions: (a) piperidine/EtOH, reflux; (b) $NaBH_4$/THF/EtOH, rt-reflux, yield: 40–50%(2 steps).



Scheme 4. Synthesis of NRA **29**–**30**. Reagents and conditions: (a) (1) KOH (40%), EtOH, 0 to rt; (b) NaOAc/ EtOH; (c) $NaBH_4$/THF/EtOHp, reflux-rt; (d) *p*-TSA, toluene, reflux, yield: 3 steps, 40% for NRA **29** and 48% for NRA **30**.

Table 1
The chemical structures of flavon-3-ols (NRA **1**–**20**) and their EC_{50} values in a cell-based NMU2R assay

Compound	R_2	R_3	R_4	R_5	R_6	R	EC_{50}
NMU							6.1 μM
Rutin							1.2 mM
NRA **1**	F	H	H	H	H	H	37.3 μM
NRA **2**	F	Cl	H	H	H	H	31.1 μM
NRA **3**	F	H	Br	H	H	H	73.0 μM
NRA **4**	F	H	H	Br	H	H	14.0 μM
NRA **5**	F	H	H	OCH_3	H	H	253.0 μM
NRA **6**	F	H	H	H	Cl	H	—
NRA **7**	F	H	H	F	Cl	H	—
NRA **8**	H	Cl	H	H	H	H	—
NRA **9**	H	Br	H	H	H	H	—
NRA **10**	H	OCH_3	H	H	H	H	—
NRA **11**	H	H	OH	H	H	H	—
NRA **12**	H	H	$N(CH_3)_2$	H	H	H	—
NRA **13**	Cl	H	Cl	H	H	H	—
NRA **14**	H	OH	OH	H	H	H	—
NRA **15**	H	OH	OCH_3	H	H	H	—
NRA **16**	H	OCH_3	OH	H	H	H	—
NRA **17**	H	H	$N(Et)_2$	H	H	H	—
NRA **18**	F	H	H	Br	H	CH_3	—
NRA **19**	F	H	H	Br	H	CH_2CH_2OH	47.0 μM
NRA **20**	F	H	H	Br	H	$CH_2COOC_2H_5$	—

2.2. Biological activity and discussion

2.2.1. Identification of flavonol derivative NMU2R agonists

Using our optimized cell-based reporter gene assay, all newly synthesized compounds were examined to determine their activities towards human NMU2R. Several cell lines expressing other GPCRs, including MC4R, MC3R, M1R, human NMU1R and MRE/CRE/SRE, were used for specificity assay.

In vitro pharmacological analysis indicated that six compounds could activate NMU2R in a dose response manner, while no compounds had effects on other GPCRs (see the Supporting information). The EC_{50} values of the six active compounds were shown in Table 1. The result showed that NRA **1**, NRA **2** and NRA **4** exhibited much higher biological activities towards NMU2R than the original identified natural product, rutin. The dose response curves of NUM, rutin, NRA **1** and NRA **4** shown in Figure 2. The EC_{50} value of NRA **4** was close to that of the endogenous ligand NMU.

2.2.2. Structure–activity relationship

On the basis of the biological activities of the newly synthesized flavonoid derivatives in the cell-based reporter gene assay, we have suggested the structure–activity relationship. As shown in Table 1, NRA **1**–**5**, all exhibit biological activity with EC_{50} values ranged from 14.0 μM to 253 μM. The common feature of the five compounds is that they all bear a 3-hydroxyl group in the C ring, and in the B ring, the 2′-position is occupied by a fluoride group, the 6′-position remains open. Besides the 2′-fluoride substituents in the B ring, the 6′-position of NRA **6** is substituted by a chloride group, consequently, NRA **6** losses its activity toward NMU2R. Introducing a 5′-fluoride in the B ring of NRA **6**, resulted in NRA **7**, exhibits no activity. As for NRA **8**–**17**, the C ring is unchanged, while the 2′-fluoride group in the B ring is eliminated, and positions 3′, 4′, and 5′ of the B ring are substituted by a range of different functional groups. The screening experiments revealed that NRA **8**–**17** exhibited no activity. SAR analysis leads to a conclusion that a 2′-fluoride group in the B ring is essential for this class of compounds (flavon-3-ols, NRA **1**–**17**) to be active toward NMU2R. By analyzing the structural differences and the corresponding EC_{50} values of the five biologically active compounds (NRA **1**–**5**), besides 2′-fluoride group in the B ring for all five compounds, NRA **1** bears no substituents in other positions of B ring and showed an EC_{50} value of 37.3 μM; NRA **2**, with 3′-chloride group, NRA **3**, with a 4′-bromide group, and NRA **4**, with a 5′-bromide group, showed EC_{50} values of 31.1 μM, 73.0 μM and 14.0 μM, respectively. Replacing the 5′-Br group in NRA **4** by a methoxyl function, leads to NRA **5**, significantly decreased the activity toward NMU2R with an EC_{50} value of 253 μM. Among the five active compounds, NRA **4** showed

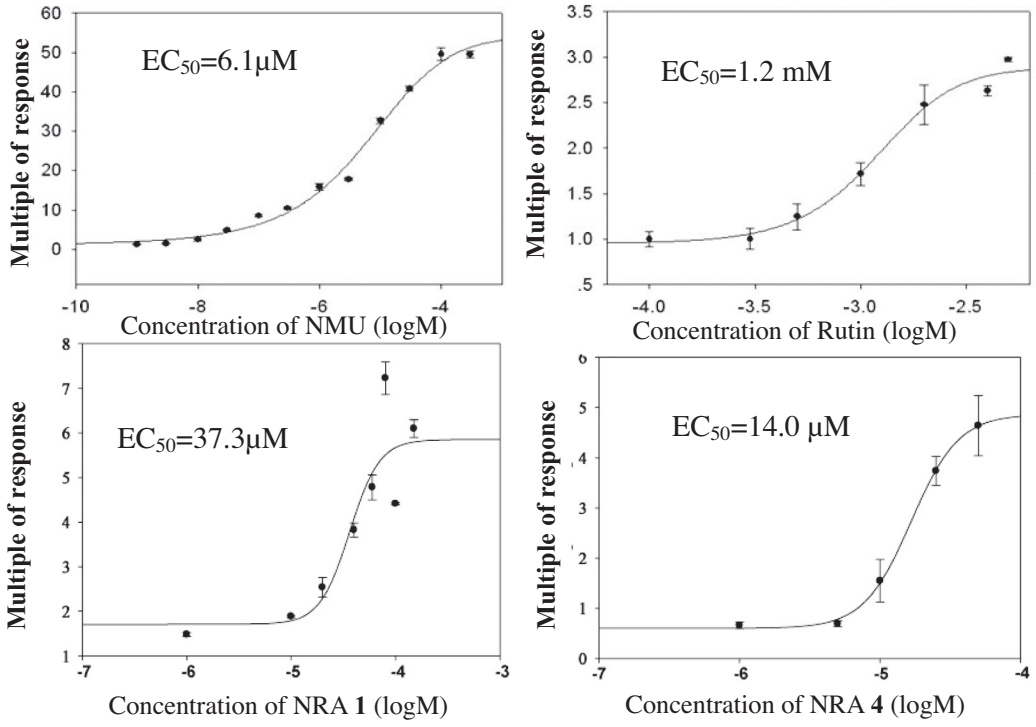


Figure 2. Response of NMU2R cell line to NUM, rutin, NRA **1** and NRA **4**. Cells were treated with endogenous ligand NMU rutin, NRA **1** and NRA **4**, the luciferase activities were measured. The data were analyzed using four-parameter logistic non-linear regression analysis method and presented as means ± SEM from three independent experiments.

the highest activity. Next, through alkylation of the 3-OH group in ring C of the most active NRA **4**, we obtained three 3-alkoxyl-substituted derivatives of NRA **4**, 3-OCH_3 (NRA **18**), $-OCH_2CH_2OH$ (NRA **19**), and $-OCH_2COOCH_2CH_3$ (NRA **20**), respectively. As shown in Table 1, NRA **18** and NRA **20** lost the biological activity, while the EC_{50} value of NRA **19** decreased to 47.0 μM from 14.0 μM of its parent NRA **4**. NRA **19**, with the 3-OH group being replaced by an $-OCH_2CH_2OH$ group, remains active toward NMU2R. This may give a notion that a free –OH in position 3 of the C ring, even as part of the substituted group, plays a critical role for this class of compounds to be active. Flavones NRA **21**–**25** and flav-3-enes NRA **26**–**30**, share the similar three ring scaffold with the flavon-3-ols (NRA **1**–**20**). However, both flavones (NRA **21**–**25**) and flav-3-enes (NRA **26**–**30**), bear no substitution groups in ring C, exhibit no biological activity toward NMU2R. Direct comparison of the structural differences between NRA **1** and NRA **21**, we can find that the two compounds share the same scaffold in rings A and B, the only structural difference comes from an 3-OH substituent in ring C (NRA **1** bears an 3-OH group, while NRA **21** does not). However, the biological activity data shows that only NRA **1** is active. This result again demonstrates that a hydroxyl function in the position 3 of ring C is essential for this class of compounds to be active toward NMU2R.

3. Conclusion

We have successfully synthesized a series of flavon-3-ols, flavone and flav-3-ene derivatives with various substitution groups aiming at the discovery of potential NMU2R agonists. In vitro study revealed that NRA **4** exhibited the strongest activity toward NMU2R in the cell-based assay. SAR analysis revealed that a structural feature of a hydroxyl group at position 3 of ring C, a 2′-fluoride group in ring B and unsubstituted 6′-position in ring B is essential for this class of compounds to be active toward NMU2R. Besides bears the above structural feature, the most potent NRA **4** bears a 5′-bromide group in the B ring. So far, we mainly discussed the halo substituent of B ring. We will introduce more functional groups, such as amino, carboxyl, amide, and trifluoromethyl into the B ring so that we could gain more insights into better active compounds and their structure–activity relationships. Now, in our lab we are researching the effect of some active compounds in animal models, and we will report the results in the future.

4. Experimental section

4.0.1. NMU2R cell-based functional assay

The functional assay for agonist screening was established as previously described[18] with further optimization. Briefly, human NMU2R cDNA was cloned using PCR method and the sequence of the cDNA clone was confirmed. The PCR product was subcloned into mammalian expression vector pcDNA3.1(+). The stable cell lines were established via co-transfection of HEK-293 cells with the reporter gene construct and human NMU2R cDNA. The cells were cultured in DMEM (Gibco) containing 600 μg/mL of G418 (Gibco) in a 37 °C incubator under an atmosphere of 5% CO_2. The confluent cells were collected using DMEM with 5% fetal bovine serum (Gibco), and the transfected cells were then seeded in a density of 4×10^4 cells/mL into wells of 96-well plates and incubated overnight. Forskolin (Sigma) and NMU were applied to the cells as positive control to evaluate the cell-based functional assay. Ten microliters of compounds at various concentrations were added to the wells and incubated at 37 °C for 7 h. One hundred microliters of Bright-Glo™ Luciferase assay reagent (Promega) was added in each well and the luciferase response was read using Analyst™ HT (Molecular Device).

4.1. General procedure for the synthesis of NRAs

All commercially chemicals and solvents are reagent grade and used without further purification. The chemicals were distilled prior to use. The reactions were carried out with the use of standard technique in nitrogen atmosphere. Reactions were monitored by thin-layer chromatography and visualized with UV light or iodine vapors. The typical work-up included washing with brine and drying the organic phase with sodium sulfate before concentration in vacuum. Final compounds were typically purified by flash chromatography on silica gel or by preparative thin-layer chromatography. Melting points were determined on a WRS-1A capillary apparatus and were uncorrected. ^{1}H NMR spectra were recorded on a Bruker DRX-500 spectrometer with tetramethylsilane as an internal reference in the indicated solvent at room temperature. Chemical shifts were reported in parts per million (ppm, δ units). Coupling constants were reported in units of hertz (Hz). Mass spectra in the electrospray ionization (ESI) mode were obtained using a micrOTOF Q mass spectrometer. Purity of all final compounds was determined by HPLC using a C18 column, and all compounds were more than 95% pure.

4.1.1. General procedure for the synthesis of NRA 1–17[20,21]

A mixture of 2-hydroxyacetophenone (1 equiv) and the corresponding aldehyde (1 equiv) in ethanol (40 mL) and 1,4-dioxane (20 mL) was cooled to 0–10 °C, 40% w/v KOH (5 equiv) solution was added dropwise. Then the reaction mixture was stirred at room temperature and monitored by TLC until the reaction completion. HCl (10%) was added to neutralize the reaction mixture. Ethyl acetate (50 mL) was then added, organic layer was separated and washed with H_2O (50 mL) and brine (50 mL), dried over anhydrous sodium sulfate and concentrated in vacuum. The residue was dissolved in 1,4-dioxane (50 mL) and ethanol (30 mL), cooled in an ice-water bath, NaOH (5.4% w/v) solution (5 equiv), 35% H_2O_2 (8 equiv) was added dropwise. The reaction mixture was stirred in an ice bath for 2 h and subsequently at room temperature overnight, resulting in a yellow suspension. After acidification with 2 M HCl to neutrality, ethyl acetate (3*50 mL) was used to extract the product, and the organic layer was washed with H_2O (50 mL) and brine (50 mL), then dried over anhydrous sodium sulfate and concentrated in vacuum. The product was purified by column chromatography. The total yields for the two steps were varied from 40% to 65%.

4.1.2. 2-(2-Fluorophenyl)-3-hydroxy-4*H*-chromen-4-one (NRA 1)

Yield 65%; mp: 120.6–121.6 °C; ^{1}H NMR ($CDCl_3$): δ 8.29–8.28 (dd, J_1 = 1.20, J_2 = 8.05, 1H), 7.82–7.79 (t, J_1 = 7.35, J_2 = 7.40, 1H), 7.73–7.69 (t, $J_1 = J_2$ = 7.89, 1H), 7.55 (d, J = 8.45, 1H), 7.53–7.49 (m, 1H), 7.45–7.43 (t, J_1 = 7.85, J_2 = 7.20, 1H), 7.32–7.30 (t, J_1 = 7.05, J_2 = 7.78, 1H), 7.26–7.23 (t, J_1 = 9.50, J_2 = 8.90, 1H), 6.67 (s, 1H); MS (M−H): $C_{15}H_8FO_3^-$ m/z calcd 255.05, found 255.05.

4.1.3. 2-(3-Chloro-2-fluorophenyl)-3-hydroxy-4*H*-chromen-4-one (NRA 2)

Yield 55%; mp: 195.8–197.7 °C. ^{1}H NMR ($CDCl_3$): δ 8.28 (d, J = 8.05, 1H), 7.74–7.70 (m, 2H), 7.58–7.54 (m, 2H), 7.46–7.43 (m, 1H), 7.27–7.24 (m, 1H), 6.68 (s, 1H); MS (M−H): $C_{15}H_7ClFO_3^-$ m/z calcd 289.01, found 289.01.

4.1.4. 2-(4-Bromo-2-fluorophenyl)-3-hydroxy-4*H*-chromen-4-one (NRA 3)

Yield 56%; mp: 156.3–161.8°C; ^{1}H NMR ($CDCl_3$): δ 8.27 (s, 1H), 7.72–7.67 (m, 2H), 7.53 (d, J = 8.50, 1H), 7.51–7.43 (m, 3H), 6.77 (s, 1H); MS (M+Na^+): $C_{15}H_8BrFO_3Na^+$ m/z calcd 356.95, found 356.95.

4.1.5. 2-(5-Bromo-2-fluorophenyl)-3-hydroxy-4*H*-chromen-4-one (NRA 4)

Yield 52%; mp: 216.8–217.6 °C; ^{1}H NMR ($CDCl_3$): δ 8.27 (d, J = 7.95, 1H), 7.94–7.93 (dd, J_1 = 2.35, J_2 = 6.00, 1H), 7.74–7.71 (t, J_1 = 7.2, J_2 = 7.25, 1H), 7.62–7.60 (m, 1H), 7.55 (d, J = 8.50, 1H), 7.46–7.43 (t, J_1 = 7.35, J_2 = 7.50, 1H), 7.15–7.12 (t, J_1 = 9.35, J_2 = 9.20, 1H), 6.71 (s, 1H); MS (M+Na$^+$): $C_{15}H_8BrFO_3Na^+$ m/z calcd 356.95, found 356.96.

4.1.6. 2-(2-Fluoro-5-methoxyphenyl)-3-hydroxy-4*H*-chromen-4-one (NRA 5)

Yield 57%; mp: 187.6–190.5 °C; ^{1}H NMR ($CDCl_3$): δ 8.29 (d, J = 8.00, 1H), 7.73–7.70 (t, J_1 = 8.30, J_2 = 7.20, 1H), 7.55 (d, J = 8.50, 1H), 7.45–7.42 (t, J_1 = 7.60, J_2 = 7.50,1H), 7.31–7.29 (m, 1H), 7.17–7.13 (t, J_1 = 9.30, J_2 = 9.40, 1H), 7.03–7.01 (m, 1H), 6.72 (br s, 1H); 3.84 (s, 3H); MS (M−H): $C_{16}H_{10}FO_4^-$ m/z calcd 285.06, found 285.06.

4.1.7. 2-(2-Chloro-6-fluorophenyl)-3-hydroxy-4*H*-chromen-4-one (NRA 6)

Yield 55%; mp: 236.4–238.4 °C. ^{1}H NMR ($CDCl_3$): δ 8.32–8.30 (dd, J_1 = 1.25, J_2 = 8.05, 1H), 7.72–7.70 (m, 1H), 7.53 (d, J_1 = 8.50,1H), 7.47–7.45 (m, 2H), 7.38 (d, J = 7.75, 1H), 7.19–7.16 (t, J_1 = 8.55, J_2 = 8.58, 1H), 6.58 (s, 1H); MS (M−H): $C_{15}H_7ClFO_3^-$ m/z calcd 289.01, found 289.00.

4.1.8. 2-(2-Chloro-3,6-difluorophenyl)-3-hydroxy-4*H*-chromen-4-one (NRA 7)

Yield 42%; mp: 232.8–234.5 °C. ^{1}H NMR ($CDCl_3$): δ 8.31 (d, J = 8.00, 1H), 7.75–7.72 (t, J_1 = 8.00, J_2 = 7.50, 1H), 7.53 (d, J = 9.00, 1H), 7.48–7.45 (t, J_1 = 7.00, J_2 = 8.00, 1H), 7.35–7.30 (m, 1H), 7.18–7.13 (m, 1H), 6.53 (s, 1H); MS (M+Na$^+$): $C_{15}H_7ClF_2O_3Na^+$ m/z calcd 330.99, found 330.99.

4.1.9. 2-(3-Chlorophenyl)-3-hydroxy-4*H*-chromen-4-one (NRA 8)

Yield 60%; mp: 105.0–106.0 °C; ^{1}H NMR ($CDCl_3$): δ 8.27–8.18 (m, 3H), 7.78–7.73 (t, J_1 = 8.50, J_2 = 7.00, 1H), 7.62 (d, J = 8.50, 1H), 7.50–7.43 (m, 3H), 7.06 (s, 1H); MS (M−H): $C_{15}H_8ClO_3^-$ m/z calcd 271.01, found 271.01.

4.1.10. 2-(3-Bromophenyl)-3-hydroxy-4*H*-chromen-4-one (NRA 9)

Yield 65%; mp: 252.2–254.0 °C; ^{1}H NMR (DMSO-d_6): δ 9.55 (s, 1H), 8.13–8.11 (t, J_1 = 12.50, J_2 = 1.00, 1H), 7.81–7.79 (m, 1H), 7.74 (d, J = 8.50, 1H), 7.68–7.65 (t, J_1 = J_2 = 8.50, 1H), 7.49–7.46 (t, J_1 = 7.00, J_2 = 8.00,2H), 7.38–7.36 (t, J_1 = J_2 = 8.00, 1H), 6.91–6.89 (m, 1H); MS (M−H): $C_{15}H_8BrO_3^-$ m/z calcd 314.96, found 314.96.

4.1.11. 2-(3-Methoxyphenyl)-3-hydroxy-4*H*-chromen-4-one (NRA 10)

Yield 55%; mp: 131.4–132.0 °C; ^{1}H NMR ($CDCl_3$): δ 8.25 (d, J = 7.45, 1H), 7.86–7.82 (m, 2H), 7.69 (d, J = 7.05,1H), 7.58 (d, J = 8.45, 1H), 7.46–7.39 (m, 2H), 7.12 (s, 1H), 7.03–7.01 (m, 1H), 3.90 (s, 3H); MS (M−H): $C_{16}H_{11}O_4^-$ m/z calcd 267.07, found 267.08.

4.1.12. 3-Hydroxyl-2-(4-hydroxyphenyl)-4*H*-chromen-4-one (NRA 11)

Yield 41%; mp: 275.6–276.4 °C; ^{1}H NMR (DMSO-d_6): δ 10.11 (s, 1H); 9.32 (s, 1H), 8.12–8.09 (t, J_1 = 10.00, J_2 = 5.00, 3H), 7.79–7.71 (m, 2H), 7.46–7.43 (t, J_1 = 5.00, J_2 = 10.00, 1H), 6.95 (d, J = 10.00, 2H), MS (M−H): $C_{15}H_8O_4^-$ m/z calcd 253.05, found 253.06.

4.1.13. 2-(4-(Dimethylamino)phenyl)-3-hydroxy-4*H*-chromen-4-one (NRA 12)

Yield 58%; mp: 190.7–191.3 °C. ^{1}H NMR ($CDCl_3$): δ 8.23–8.16 (m, 3H), 7.64–7.61 (t, J_1 = J_2 = 7.50, 1H), 7.53 (d, J = 8.50,1H), 7.37–7.35 (t, J_1 = 7.00, J_2 = 7.50, 1H), 6.90 (s, 1H), 6.77 (d, J = 8.00, 2H), 3.04 (s, 6H); MS (M+Na$^+$): $C_{17}H_{15}NO_3Na^+$ m/z calcd 304.09, found 304.09.

4.1.14. 2-(2,4-Dichlorophenyl)-3-hydroxy-4*H*-chromen-4-one (NRA 13)

Yield 59%; mp: 211.9–213.7 °C; ^{1}H NMR (DMSO-d_6): δ 9.48 (s, 1H), 8.17–8.15 (dd, J_1 = 1.25, J_2 = 8.00, 1H), 7.85 (d, J = 1.95, 1H), 7.80 (d, J = 1.30, 1H), 7.75 (d, J = 8.30, 1H), 7.66–7.61 (m, 2H), 7.51 (d, J = 7.15, 1H); MS (M+Na$^+$): $C_{15}H_8Cl_2O_3Na^+$ m/z calcd 328.97, found 328.98.

4.1.15. 2-(3,4-Dihydroxyphenyl)-3-hydroxy-4*H*-chromen-4-one (NRA 14)

Yield 40%; mp: 274.0–274.8 °C; ^{1}H NMR (DMSO-d_6): δ 9.57 (s, 1H), 9.28 (d, J = 6.50, 2H), 8.09 (d, J = 8.00, 1H), 7.79–7.71 (m, 2H), 7.70 (d, J = 8.00, 1H), 7.61–7.60 (t, J_1 = 1.50, J_2 = 8.50, 1H), 7.47–7.44 (t, J_1 = 7.50, J_2 = 15.00, 1H), 6.90 (d, J = 8.50, 1H); MS (M−H): $C_{15}H_9O_5^-$ m/z calcd 269.04, found 269.05.

4.1.16. 3-Hydroxy-2-(3-hydroxy-4-methoxyphenyl)-4*H*-chromen-4-one (NRA 15)

Yield 45%; mp: 206.9–207.8 °C; ^{1}H NMR (DMSO-d_6): δ 9.33 (s, 2H), 8.11–8.09 (dd, J_1 = 1.35, J_2 = 7.95, 1H), 7.88–7.71 (m, 4H), 7.47–7.44 (m, 1H), 7.10 (d, J = 8.65, 1H), 3.85 (s, 3H); MS (M−H): $C_{16}H_{11}O_5^-$ m/z calcd 283.06, found 283.06.

4.1.17. 3-Hydroxy-2-(4-hydroxy-3-methoxyphenyl)-4*H*-chromen-4-one (NRA 16)

Yield 45%; mp: 244.0–244.6 °C; ^{1}H NMR (DMSO-d_6): δ 9.73 (s, 1H), 9.36 (s, 1H), 8.10 (d, J = 7.75, 1H), 7.83–7.76 (m, 4H), 7.47–7.44 (m, 1H), 6.96 (d, J = 8.45, 1H), 3.86 (s, 3H); MS (M−H): $C_{16}H_{11}O_5^-$ m/z calcd 283.06, found 283.07.

4.1.18. 2-(4-(Diethylamino)phenyl)-3-hydroxy-4*H*-chromen-4-one (NRA 17)

Yield 56%; mp: 115.6–118.4 °C; ^{1}H NMR ($CDCl_3$-d_6): δ 8.23 (d, J = 8.00, 1H), 8.17 (d, J = 9.00, 2H), 7.65–7.63 (m, 1H), 7.39–7.36 (t, J_1 = 7.00, J_2 = 8.00, 1H), 7.55 (d, J = 8.50, 1H), 6.89 (s, 1H), 6.77 (d, J = 9.00, 2H), 3.47–3.43 (q, J_1 = 7.00, J_2 = 14.00, 4H), 1.26–1.21 (t, J_1 = 7.00, J_2 = 7.50, 6H); MS (M+Na$^+$): $C_{19}H_{19}NO_3Na^+$ m/z calcd 332.13, found 332.13.

4.2. General procedure for the synthesis of NRA 18–20

A mixture of NRA **4** (1 equiv), potassium carbonate (2.5 equiv) and the corresponding bromides (1 equiv) in DMF (50 mL) was stirred at room temperature and the reaction was monitored by TLC. After the reaction completion, HCl (10%) was employed to neutralize the reaction mixture and then ethyl acetate (50 mL) was added, the organic layer was washed with H_2O (50 mL) and brine (50 mL), dried over anhydrous sodium sulfate and concentrated in vacuum. The product was purified by column chromatography, the yield was in a range of 90∼95%.

4.2.1. 2-(5-Bromo-2-fluorophenyl)-3-methoxy-4*H*-chromen-4-one (NRA 18)

Yield 95%; mp: 111.8–112.9 °C; ^{1}H NMR ($CDCl_3$): δ 8.28 (d, J = 8.00, 1H), 7.78–7.76 (dd, J_1 = 2.25, J_2 = 5.75, 1H), 7.71–7.68 (t, J_1 = 7.30, J_2 = 15.55, 1H), 7.62–7.60 (m, 1H), 7.49 (d, J = 8.45, 1H), 7.44–7.41 (t, J_1 = 7.85, J_2 = 15.05, 1H), 7.15–7.11 (m, 1H), 3.95 (s, 3H); MS (M+Na$^+$): $C_{16}H_{10}BrFO_3Na^+$ m/z calcd 370.97, found 370.98.

4.2.2. 2-(5-Bromo-2-fluorophenyl)-3-(2-hydroxyethoxy)-4*H*-chromen-4-one (NRA 19)

Yield 90%; mp: 121.1–122.1 °C; ^{1}H NMR ($CDCl_3$): δ 8.30 (d, J = 8.05, 1H), 7.83–7.81 (m, 1H), 7.72 (d, J = 8.35, 1H), 7.65–7.64

(m, 1H), 7.52 (d, J = 8.50, 1H), 7.48–7.45 (t, J_1 = 7.35, J_2 = 15.10, 1H), 7.17–7.13 (t, J_1 = 9.20, J_2 = 18.25, 1H), 4.08–4.06 (t, J_1 = 4.00, J_2 = 8.15, 2H), 3.80–3.79 (t, J_1 = 4.00, J_2 = 7.85, 2H); MS (M+Na$^+$): $C_{17}H_{12}BrFO_4Na^+$ m/z calcd 400.98, found 400.98.

4.2.3. Ethyl 2-(2-(5-bromo-2-fluorophenyl)-4-oxo-4*H*-chromen-3-yloxy)acetate (NRA 20)

Yield 93%; mp: 79.8–82.1 °C; ^{1}H NMR ($CDCl_3$): δ 8.19 (d, J = 8.00, 1H), 7.81–7.80 (m, 1H), 7.64–7.62 (t, J_1 = 0.90, J_2 = 8.35, 1H), 7.55–7.54 (t, J_1 = 4.35, J_2 = 8.75, 1H), 7.44 (d, J = 8.50, 1H), 7.37–7.34 (m, 1H), 7.08–7.04 (t, J_1 = 9.30, J_2 = 18.35, 1H), 4.89 (s, 2H), 4.16–4.12 (m, 2H), 1.20–1.17 (t, J_1 = 7.10, J_2 = 14.20, 3H); MS (M+H): $C_{19}H_{15}BrFO_5^+$ m/z calcd 421.01, found 421.01.

4.2.4. General procedure for the synthesis of NRA 21–25[22,23]

To an ice-cooled 2-hydroxyacetophenone (1 equiv) and the corresponding aldehyde (1 equiv) solution in EtOH (70 mL), 40% KOH (6 equiv) was added. The mixture was stirred under nitrogen atmosphere at ambient temperature until reaction was completed. Thereafter, the reaction mixture was slowly poured into excess 6 N HC1, extracted with ethyl acetate (50 mL) three times and washed with H_2O and brine. Dried over anhydrous sodium sulfate and concentrated in vacuum. The crude product 2′-hydroxychalcone was passed through a fast column and then used in the next step.

To a solution of the corresponding 2′-hydroxychalcone (1 equiv) in DMSO (20.0 mL), I_2 (0.01 equiv) was added. The mixture was heated at reflux for 1 h. Then the reaction mixture was poured into water and extracted with ethyl acetate (3*25.0 mL). The organic layer was washed with water and brine, dried over anhydrous sodium sulfate. The solvent was evaporated in vacuum and the residue was purified by column chromatography, the combined total yield for the two steps ranged from 40% to 65%.

4.2.5. 2-(2-Fluorophenyl)-4*H*-chromen-4-one (NRA 21)

Yield 65%; mp: 100.4–100.7 °C; ^{1}H NMR ($CDCl_3$): δ 8.24–8.22 (dd, J_1 = 1.60, J_2 = 7.95, 1H), 7.93 (d, J = 1.65, 1H), 7.70–7.69 (t, J_1 = 1.20, J_2 = 8.25, 1H), 7.54 (d, J = 8.60, 2H), 7.44–7.41 (t, J_1 = 7.80, J_2 = 15.05, 1H), 7.34–7.31 (t, J_1 = 7.65, J_2 = 15.25, 1H), 7.26–7.22 (m, 1H), 6.94 (s, 1H); MS (M+H): $C_{15}H_{10}FO_2^+$ m/z calcd 241.07, found 241.07.

4.2.6. 2-(4-Hydroxyphenyl)-4*H*-chromen-4-one (NRA 22)

Yield 40%; mp: 258.5–262.4 °C; ^{1}H NMR (DMSO-d_6): δ 10.30 (br s, 1H), 8.03 (d, J = 8.00, 1H), 7.97 (d, J = 8.50, 2H), 7.83–7.80 (m, 1H), 7.75 (d, J = 8.50, 1H), 7.50–7.47 (t, J_1 = 7.00, J_2 = 7.50, 1H), 6.94 (d, J = 8.50, 2H), 6.87 (s, 1H); MS (M−H): $C_{15}H_9O_3^-$ m/z calcd 237.06, found 237.06.

4.2.7. 2-(2,4-Dichlorophenyl)-4*H*-chromen-4-one (NRA 23)

Yield 58%; mp: 124.1–125.1 °C; ^{1}H NMR ($CDCl_3$): δ 8.29–8.27 (dd, J_1 = 1.00, J_2 = 8.00, 1H), 7.73–7.72 (t, J_1 = 3.00, J_2 = 1.00, 1H), 7.63–7.59 (m, 2H), 7.53 (d, J = 9.00, 1H), 7.49–7.42 (m, 2H), 6.67 (s, 1H); MS (M+Na$^+$): $C_{15}H_8Cl_2O_2Na^+$ m/z calcd 312.98, found 312.98.

4.2.8. 2-(3-Hydroxy-4-methoxyphenyl)-4*H*-chromen-4-one (NRA 24)

Yield 45%; mp: 192.2–194.9 °C; ^{1}H NMR (DMSO-d_6): δ 9.48 (s, 1H), 8.05–8.03 (dd, J_1 = 1.50, J_2 = 7.85, 1H), 7.82–7.80 (dd, J_1 = 1.30, J_2 = 6.95, 1H), 7.43 (d, J = 3.35, 1H), 7.59–7.57 (dd, J_1 = 1.75, J_2 = 8.50, 1H), 7.50–7.47 (dd, J_1 = 7.20, J_2 = 12.05, 2H), 7.10 (d, J = 8.60, 1H), 6.83 (s, 1H), 3.87 (s, 3H); MS (M−H): $C_{16}H_{11}O_4^-$ m/z calcd 267.06, found 267.07.

4.2.9. 2-(4-Hydroxy-3-methoxyphenyl)-4*H*-chromen-4-one (NRA 25)

Yield 48%; mp: 191.4–192.1 °C; ^{1}H NMR ($CDCl_3$): δ 8.22 (d, J = 7.85, 1H), 7.69–7.66 (t, J_1 = 7.20, J_2 = 7.25, 1H), 7.56–7.51 (m, 2H), 7.42–7.37 (m, 2H), 7.05 (d, J = 8.35, 1H), 6.74 (s, 1H), 6.39 (s, 1H), 3.99 (s, 3H); MS (M−H): $C_{16}H_{11}O_4^-$ m/z calcd 267.07, found 267.07.

4.3. General procedure for the synthesis of NRA 26–28

2-Hydroxyacetophenone (1 equiv) and the corresponding aldehyde **2** (1 equiv) were dissolved in ethanol (30 mL) and piperidine (5 equiv) was added. The mixture was heated to reflux until the starting material disappeared (monitored by TLC). The reaction mixture was then cooled to room temperature and evaporated the solvent in vacuum. The residue was dissolved in THF (40 mL) and ethanol (20 mL), and $NaBH_4$ (1 equiv) was slowly added to the solution. The mixture was heated to reflux and monitored by TLC until disappearance of the starting material. The product was purified through column chromatography, the total yield was in the range of 40∼50%.

4.3.1. 2-(4-Methoxyphenyl)-2*H*-chromene (NRA 26)

Yield 50%; mp: 131.5–134.1 °C; ^{1}H NMR ($CDCl_3$): δ 7.51 (d, J = 7.15, 1H), 7.37 (d, J = 8.60, 2H), 7.20 (m, 1H), 7.00–6.97 (t, J_1 = 7.50, J_2 = 7.91, 1H), 6.93 (d, J = 8.70, 2H), 6.90–6.87 (t, J_1 = 7.30, J_2 = 6.50, 2H), 5.12–5.07 (m, 2H), 3.83 (s, 3H); MS (M+HCOO$^-$): $C_{17}H_{15}O_4^-$ m/z calcd 283.08, found 283.01.

4.3.2. 4-(2H-chromen-2-yl)-N,N-dimethylbenzenamine (NRA 27)

Yield 55%; mp: 76.9–77.7 °C; ^{1}H NMR ($CDCl_3$): δ 7.34 (d, J = 8.0 Hz, 2H), 7.11–7.08 (t, J_1 = 7.00, J_2 = 8.00, 1H), 7.02 (d, J = 7.50, 1H), 6.87–6.84 (t, J_1 = 7.00, J_2 = 7.50, 1H), 6.77–6.72 (m, 3H), 6.55 (d, J = 9.50, 1H), 5.85–5.79 (m, 2H), 2.95 (s, 6H); MS (M+H): $C_{17}H_{18}NO^+$ m/z calcd 252.14, found 252.14.

4.3.3. 5-(2*H*-chromen-2-yl)-2-methoxyphenol (NRA 28)

Yield 40%; mp: 187.1–187.7 °C; ^{1}H NMR ($CDCl_3$): δ 7.51 (d, J = 7.05, 1H), 7.22–7.18 (t, J_1 = 9.00, J_2 = 7.40, 1H), 7.03 (s, 1H), 6.99–6.93 (m, 2H), 6.89–6.86 (m, 2H), 5.65 (s, 1H), 5.11–5.06 (m, 2H), 3.90 (s, 3H); MS (M+Na$^+$): $C_{16}H_{14}O_3Na^+$ m/z calcd 277.08, found 277.09.

4.4. General procedure for the synthesis of NRA 29 and NRA 30[24]

To an ice-cooled solution of 2-hydroxyacetophenone (1 equiv) and the corresponding aldehyde (1 equiv) in EtOH (70 mL) was added 40% KOH (6 equiv). The solution was stirred under nitrogen atmosphere at ambient temperature until reaction was complete (monitored by TLC). Thereafter, the reaction mixture was slowly poured into excess 6 N HC1, extracted with ethyl acetate (50 mL) three times and washed with H_2O and brine, dried over anhydrous sodium sulfate and concentrated in vacuum. The crude product 2′-hydroxychalcone was purified in column chromatography.

The purified product was dissolved in EtOH (40 mL), and sodium acetate (3.0 equiv) was added. The mixture was refluxed and monitored by TLC. After the reaction complete, the mixture was cooled to room temperature, and powdered sodium borohydride (0.5equiv) was added in portions. The mixture was stirred for additional 1.5 h, and quenched by dropwise addition of acetic acid. The mixture was then poured into ice–water (100 mL) and extracted with ethyl acetate (50 mL) three times and washed with H_2O and brine. The organic layer was dried over anhydrous sodium sulfate and concentrated in vacuum. The crude mid product was passed through a fast column and subjected to next step.

A mixture of *p*-toluenesulfonic acid (cat) and the crude product in toluene (80 mL) was heated to reflux for 2 h. The reaction mixture was cooled to room temperature and washed with saturated sodium bicarbonate solution (25 mL*2). The toluene layer was dried over anhydrous sodium sulfate and the solvent was evaporated under vacuum. The product was purified through column chromatography.

4.4.1. 2-(2-Fluorophenyl)-2*H*-chromene (NRA 29)

Yield 40%; ^{1}H NMR ($CDCl_3$): δ 7.57–7.56 (m, 1H), 7.17–7.16 (m, 1H), 7.18–7.12 (m, 3H), 7.06–7.04 (dd, J_1 = 0.15, J_2 = 7.50, 1H), 6.94–6.92 (m, 1H), 6.86 (d, J = 8.00, 1H), 6.59–6.57 (dd, J_1 = 1.50, J_2 = 10.00, 1H), 6.34–6.33 (m, 1H), 5.84–5.82 (dd, J_1 = 3.50, J_2 = 9.75, 1H); MS (M−H): $C_{15}H_{10}FO^-$ *m*/*z* calcd 225.07, found 225.07.

4.4.2. 2-(2,4-Dichlorophenyl)-2*H*-chromene (NRA 30)

Yield 48%; ^{1}H NMR ($CDCl_3$): δ 7.51 (d, J = 8.40, 1H), 7.39 (d, J = 2.00, 1H), 7.22–7.20 (dd, J_1 = 2.00, J_2 = 8.40, 1H), 7.12 (m, 1H), 7.00–6.99 (dd, J_1 = 1.30, J_2 = 7.40, 1H), 6.88 (m, 1H), 6.80 (d, J = 8.05, 1H), 6.51 (d, J = 9.05, 1H), 6.30–6.29 (t, J_1 = 3.40, J_2 = 2.10, 1H), 5.75–5.72 (dd, J_1 = 3.35, J_2 = 9.85, 1H); MS (M+HCOO$^-$): $C_{16}H_{11}Cl_2O_3^-$ *m*/*z* calcd 321.01, found 320.98.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2014.08.038.

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