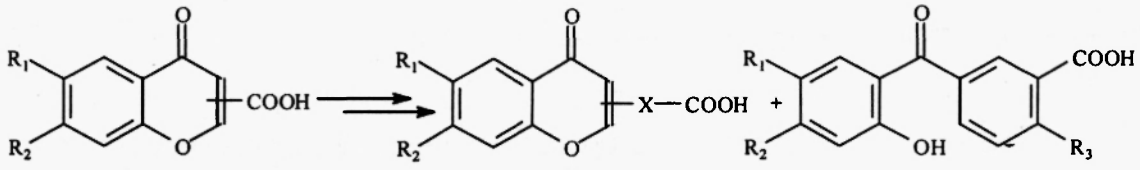
THE SYNTHESIS AND PRELIMINARY EVALUATION OF SUBSTITUTED CHROMONES, COUMARINS, CHROMANONES, AND BENZOPHENONES AS RETINOIC ACID RECEPTOR LIGANDS

Shyam Desai[1], Weilin Sun[1], Jerome Gabriel[2] and Daniel J Canney*[,1]
[1]Department of Pharmaceutical Sciences, School of Pharmacy, Temple University, 3307 North Broad Street, Philadelphia, PA 19140 [2]Department of Biochemistry, School of Medicine, Temple University
E-mail : canney@temple.edu, 1-215-707-6924

Abstract : Utilizing molecular modeling techniques and structure-activity relationship data from the literature, a series of 2- and 3-substituted chromones and related heterocycles have been designed and synthesized as retinoic acid receptor ligands. The compounds were prepared using known coupling reactions and Wittig-Horner-Emmons reaction conditions. These compounds were then evaluated for affinity to retinoic acid receptor subtypes. Several of the compounds reported herein were found to bind with moderate affinity to the target receptors.



R_1 = iPr, R_2 = H; R_1, R_2 = $(CH_3)_2CH(CH_2)_2CH(CH_3)_2$ or H; R_3 = H or Me

Introduction

Retinoid receptors are a group of transcription factors that belong to the nuclear hormone receptor super-family. There are two kinds of nuclear retinoid receptors; retinoic acid receptors (RARs; α, β and γ) and retinoid X receptors (RXRs; α, β and γ). RARs and RXRs are transcriptional regulators whose activity is mediated by their ligand binding domain. Endogenous ligands include all-trans retinoic acid (ATRA; selective for RAR) and 9-cis-retinoic acid (binds both RXRs and RARs). The ability of retinoids to regulate cell proliferation and differentiation has led to their clinical use in the treatment of dermatological disorders and certain forms of cancer .[1,3] While the available retinoids are therapeutically useful drugs, they are associated with toxicity that may be due, in part, to their nonselective nature. Compounds that bind selectively to one subtype of RARs (alpha, beta, or gamma) may exhibit fewer adverse effects.

The pharmacophore is a useful concept when designing novel ligands for receptors of interest. The pharmacophore is a term used to describe the minimal structural requirements necessary for a molecule to bind to a specific receptor. In the case of RAR ligands, a very general pharmacophoric model has been proposed .[4,5] The proposed model divides ATRA into a hydrophobic region (A), a linker or tether region (B) and a hydrophilic region (C) (see Figure-1).

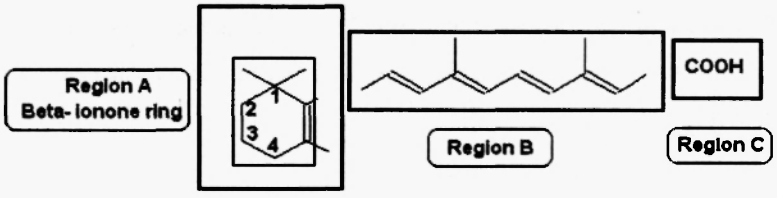


Figure-1 : Pharmacophoric Model for RARs ligands based on ATRA .[4]

This model is quite general compared to pharmacophoric models established for other receptor ligands e.g., Nicotinic Ach receptor;[6,7] and has limited usefulness in directing the design of new ligands. A goal of the present work was to use molecular modeling techniques in conjunction with SAR data in the literature to design novel, heterocycle-containing ligands for RAR receptors. The ligands will contribute to the SAR data for RAR ligands and may help to further develop the model to include factors such as optimal inter-atomic distance between key functional groups and the need for planarity within the molecules.

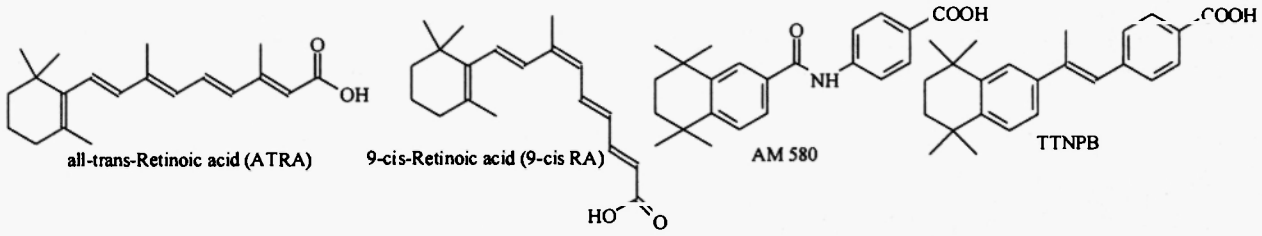
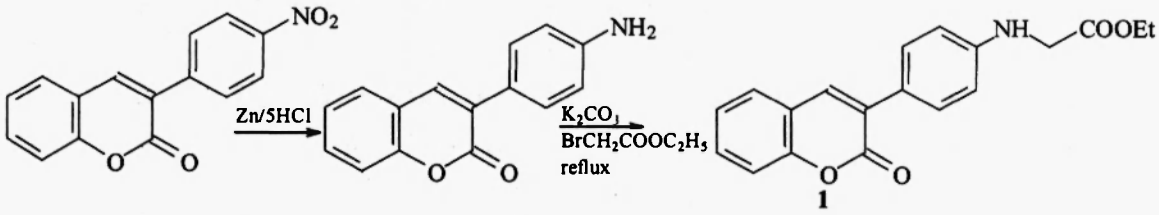


Figure-2 : Structures of ATRA, 9-cis RA, AM580 (RARα agonist) and Rexiniods.

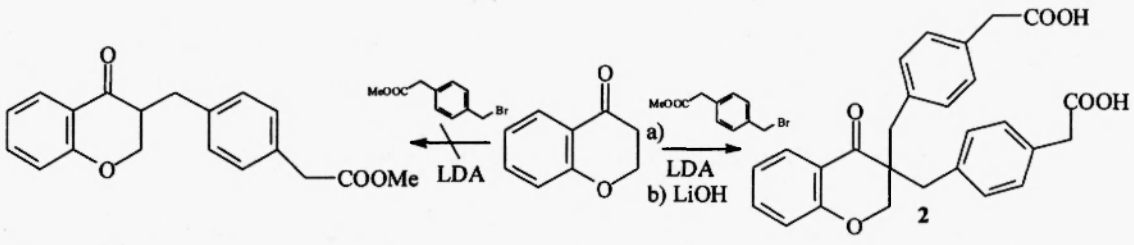
Chromone- and flavone-based compounds have been reported to bind nuclear receptors including the Aromatic Hydrocarbon Receptors (AHRs) and RARs.[8-11] These reports, as well as, the planarity of the nucleus prompted us to design chromone-based and related compounds as potential RAR ligands. We herein report the design, synthesis and preliminary evaluation of structurally diverse heteroatom-containing ligands for RARs. The compounds designed using molecular modeling techniques and SAR data were prepared using standard coupling techniques, as well as, Wittig-Horner-Emmons reaction conditions. An unexpected rearrangement occurred in the course of Wittig-Horner-Emmons reactions leading to benzophenone-based ligands. The compounds were evaluated in radioligand binding assays ([^{3}H] ATRA) and several compounds were found to have moderate affinity for the receptors.

Chemistry

The synthesis of coumarin-based compound **1** is shown in Scheme 1. Using 3-(4-nitrophenyl)-2*H*-chromen-2-one as starting material, the nitro group was reduced to the corresponding amine in quantitative yield with activated Zn powder in HCl/methanol solution. The amine was alkylated with ethyl bromoacetate in a refluxing suspension of potassium carbonate in acetone. A mono-substituted ligand containing the chromanone system was designed as a potential RAR ligand. The attempted synthesis of compound **2** is shown in Scheme 2. The chromanone was treated with lithium diisopropylamide (LDA) and ethyl 4-(bromomethyl)phenyl acetate added dropwise with stirring (the ester was prepared in acidic methanol solution). ^{1}H NMR and ^{1}H COSEY data revealed that diester of compound **2** was the only product. The diester shown in Scheme-2 was isolated and hydrolyzed under basic conditions to provide compound **2** for testing in the binding assay.

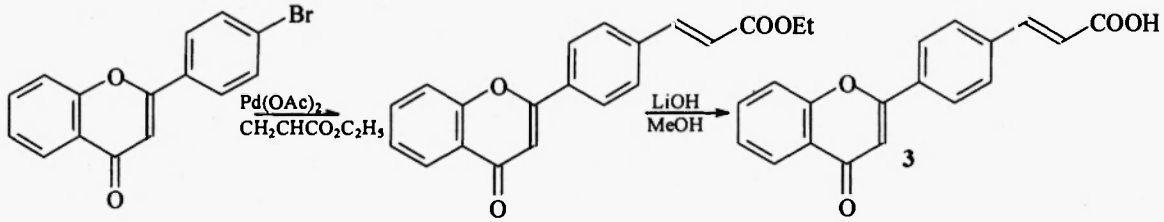


Scheme-1 : Synthetic route used for the preparation of compound 1.



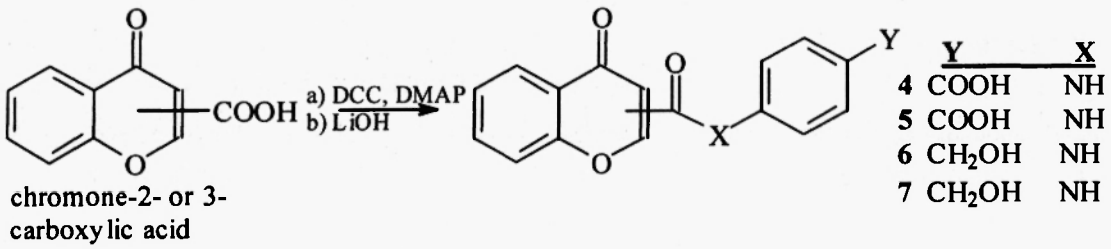
Scheme-2 : Synthetic route used for the preparation of compound 2.

The synthesis of flavone-based compound **3** is shown in Scheme 3 and involves standard Heck reaction conditions. Hence, 4'-bromoflavone was coupled to ethyl acrylate[12] and the resulting ester hydrolyzed under alkaline conditions (LiOH in methanol) to provide **3** in moderate yield.



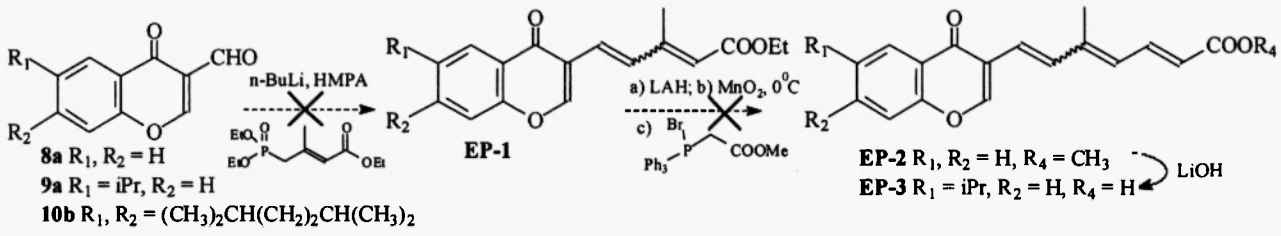
Scheme-3 : Synthetic route used for the preparation of compound 3.

Based on the well known RAR ligand, AM580, a series of compounds in which methyl benzoates and benzyl alcohols were coupled to the chromone nucleus via an amide linker. The 2– and 3–substituted chromones were prepared using standard coupling reactions involving DDC and the synthesis is shown in Scheme-4.



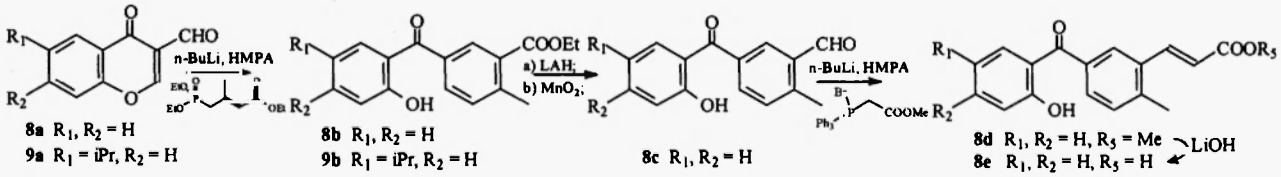
Scheme-4 : Synthetic route used for the preparation of compound 4 – 7.

A series of ligands containing all-trans poly-olefinic chains coupled to the chromone nucleus were designed to resemble ATRA. The synthesis of target ligands containing an olefinic tether region coupled to a chromone ring could be envisioned coming from a variety of sources, the majority of which involved Wittig and/or Wadsworth-Horner-Emmons (WHE) reaction conditions. The attempted synthesis of the proposed compounds is shown in Scheme-5.

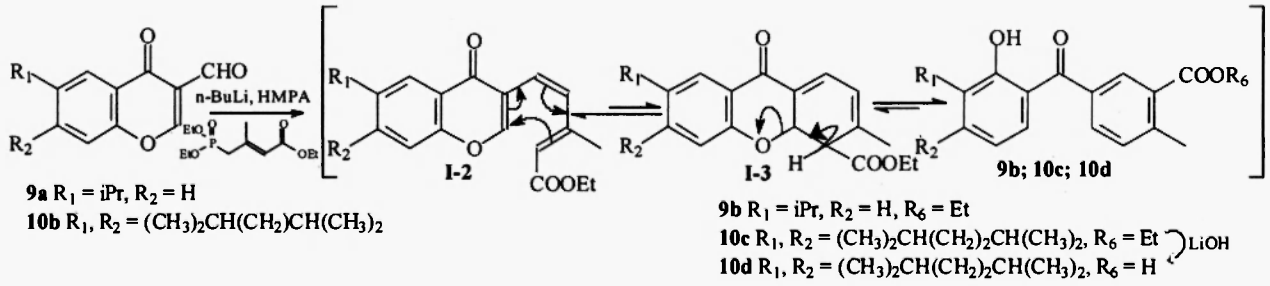


Scheme-5 : Expected Products {EP} of Wittig and W-H-E reactions.

However, an unanticipated rearrangement occurred during the course of the reaction that resulted in the isolation benzophenone-based ligands. The synthesis of the products **8e, 9b** and **10d**[9, 13-16] isolated and tested is shown in Schemes-6 and 7. A proposed mechanism for the rearrangement of these compounds has been reported previously as shown in Scheme 7.[16]



Scheme-6 : Synthesis of compounds 8e and 9b.



Scheme-7 : Rearrangement of the chromone starting materials to the corresponding benzophenones.

Results and Discussion

Molecular modeling studies and structure-activity relationship data from the literature were used to design a series of heterocyclic compounds as potential ligands for RARs. Table 1 shows the structure of compounds designed, synthesized and tested in the current work. The chromone–based ligands possess minimal bulk and include heteroatoms in the ring systems of Region A. The linker region of the compounds includes amides, cinnamates, anilines and tolyl groups. Modeling studies suggested that these chromone-based ligands fit the pharmacophoric model for RAR ligands and were more planar and less bulky than previous test compounds prepared in our lab. This study

showed 6 had similar spatial orientation to AM580 and was also in the proximity of essential AAs like serine 232. The first series of compounds designed and synthesized here (compounds 1 – 7) were screened in RAR binding assays and exhibited no affinity for RARs. With the exception of 2 (disubstituted compound) the compounds met the pharmacophoric criteria proposed in the earlier model and resembled ATRA in docking experiments.

Table-1 : Inter-atomic distances (IADs) & binding data for RAR subtypes.

Compound No.	IADs (Å)[a]	Binding RAR (s) (%)	Compound No.	IADs (Å)[a]	Binding RAR (s) (%)[b]
1	12.2- 13.8	NB[c]	6	13.7-13.9	NB
2	11.9- 12.3	NB	7	12.2	NB
3	13.6- 13.9	NB	8e	9.0-9.5	NB
4	13.4-13.6	NB	9b	9.1-9.7	NB
5	13.67	NB	10d	8.78- 9.84	22%[d]

a. Inter-atomic Distances (IADs) measured in Å performed by Dr. Gabriel. b. % inhibition of specific binding at 500nM. RAR binding studies performed by Dr. Soprano, Biochemistry Dept., School of Medicine. c. NB – No binding affinity for RARs.

Based on the negative results obtained for the compounds it was necessary to modify the criteria used for ligand design. Therefore, the chromone nucleus was retained but tether Region B was modified to incorporate poly-olefinic groups. The compounds thus modified more closely mimicked the structure of known RAR ligands and ATRA. Compounds 8e, 9b and 10d were designed as chromone–based ligands with poly–olefinic side chains to resemble ATRA (Scheme 5). However, the expected products of the Wittig-Horner-Emmons reactions were not isolated and instead, benzophenones were obtained. The synthetic details and proof of the rearrangement has been reported elsewhere.[16] The x–ray crystal structure of compound 10d is shown in Figure-3 and clearly demonstrates the benzophenone nucleus in the compound. The benzophenone–based ligands were also tested in binding assays and found to bind with low to moderate affinity to RAR receptors.

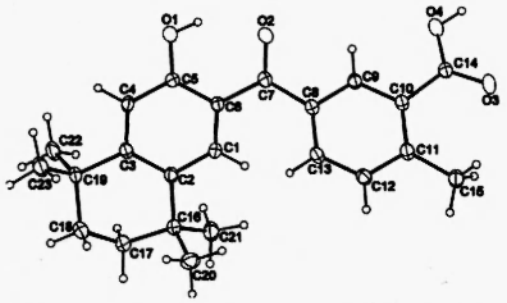


Figure-3 : ORTEP representation of crystal structure of compound 10d with 30% probability thermal ellipsoids.[16]

Experimental

General : Reactions were carried out in oven-dried glassware under a nitrogen atmosphere and were magnetically stirred and monitored by thin-layer chromatography (TLC) with Analtech 250 micron pre-coated silica gel plates. Dichloromethane (CH_2Cl_2) distilled from CaH_2 and Tetrahydrofuran (THF) were freshly distilled from Na/benzophenone under N_2. Except as otherwise indicated, all reagents were purchased and used without purification. Flash column chromatography was performed using silica gel 60 (particle size 230-400 mesh) supplied by Aldrich and SiliCycle Inc. Yields refer to chromatographically pure compounds. NMR spectra were recorded on a Bruker Avance 400 at 400MHz for ^1H NMR (some on GE at 300 MHz and 500 MHz) and 100MHz for ^{13}C NMR. Chemical shifts were reported relative to solvents or an internal standard (TMS). X-ray crystallographic analysis was performed at Dept of Chem, Univ. of PA.

Ethyl [4-(2-oxo(2*H*)chromen-3-yl)phenyl]aminoacetate (1)
To a methanolic suspension (15 mL) of 3-(4-nitrophenyl)-2*H*-chromen-2-one (0.5 g, 1.9 mmol) was added 2.0 g of freshly activated Zn and 2 mL of 5% HCl. The mixture turned yellow and was stirred at rt. After 2 h, the mixture was filtered, concentrated under reduced pressure and purified by flash chromatography (silica gel; 1:1, hexane: EtOAc) to afford 3-(4-aminophenyl)-2*H*-chromen-2-one (0.44 g, 100%) as a yellow solid: ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.01 (s, 1H), 7.65-7.63 (d, J = 7.7 Hz, 1H), 7.53-7.51 (d, J = 8.7 Hz, 2H), 7.48-7.45 (d, J = 7.1 Hz, 1H), 7.31-7.28 (m, 2H), 8.84-6.82 (d, J = 8.7 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.5, 160.3, 152.9, 152.7, 138.2, 131.2, 129.1, 128.4, 127.3, 125.7, 124.7, 120.1, 115.9, 112.6. To a solution of 3-(4-aminophenyl)-2*H*-chromen-2-one (200 mg, 0.8 mmol) in acetone (20 mL) was added 0.1 g of dry potassium carbonate and ethyl bromoacetate (0.2 mL, 1.8 mmol). The resulting mixture was heated under reflux for 6 h then stirred at rt overnight. The mixture was filtered, concentrated and purified by flash chromatography (silica gel; 1:1, hexane: EtOAc) to yield **1** (110 mg; yield: 81% , 100 mg of 3-(4-aminophenyl)-2*H*-chromen-2-one was recovered) as a yellow solid: ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.68 (s, 1H), 7.56-7.53 (d, 2H, J = 9.4 Hz), 7.45-7.38 (m, 2H), 7.26 (d, 1H, J = 8.0), 7.22-7.18 (t, 1H, J = 8.4 Hz), 6.60 (d, 2H, J = 9.5 Hz), 4.18 (q, 2H), 3.87(s, 2H), 1.25 (t, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.2, 161.4, 153.5, 147.9, 137.7, 137.6, 131.0, 130.1, 128.5, 127.9, 124.7, 124.6, 120.5, 116.7, 113.1, 61.9, 45.9, 14.6.

3,3-Bis[4-(carboxymethyl)phenylmethyl]-4-oxochroman (2)
Dry HCl gas was bubbled through a solution of 4-(bromomethyl)phenyl acetic acid (0.7g, 3.1 mmol) in MeOH (20 mL). The resulting mixture was stirred at 40 °C for 48 h and then concentrated under reduced pressure and purified by flash chromatography (silica gel; 10:1 to 3:1, hexane: EtOAc) to afford methyl [4-(bromomethyl)phenyl] acetate (0.55g, 74%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.26 (d, J = 8.1 Hz, 2H), 7.19 (d, J = 8.0 Hz, 2H), 4.49 (s, 2H), 3.61 (s, 3H), 3.55 (s, 2H). To a solution of 4-chromanone (0.3g, 2.0 mmol) in dry THF (10 mL) at -78 °C was added LDA (1.75 mL, 1.8 M in hexane, 3.1 mmol). The mixture was stirred for 15 min and a solution of methyl [4-(bromomethyl)phenyl] acetate in dry THF (10 mL) was added dropwise. The mixture was stirred at -78 °C for 4 h, allowed to warm to rt, stirred overnight, quenched with saturated NH_4Cl (50 mL) and extracted with EtOAc (50 mL x 2). The combined organic layers were washed with brine (30 mL), dried over $MgSO_4$ and purified by flash chromatography (silica gel; 3:1, hexane: EtOAc) to provide dimethyl ester of **2** (0.36 g, 37%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.88-7.86 (dd, 1H, J = 1.6, 6.2 Hz), 7.37-7.35 (td, 1H, J = 1.4, 6.8 Hz), 7.09 (d, 4H, J = 8.1 Hz), 7.35 (d, 4H, J = 8.2 Hz), 6.96-6.92 (t, 1H, J = 8.0 Hz), 6.87 (d, 1H, J = 8.3 Hz), 4.00 (s, 2H), 3.60 (s, 6H), 3.50 (s, 4H), 3.25 (d, 2H, J = 13.6 Hz), 2.48 (d, 2H, J = 13.6 Hz).^{13}C NMR (100 MHz, $CDCl_3$): δ 195.7, 172.4, 161.4, 136.2, 135.2, 132.9, 131.4, 129.6, 128.4, 121.9, 120.9, 118.2, 77.8, 77.5, 77.2, 71.8, 53.0, 52.5, 51.9, 50.8, 41.7, 41.1, 40.6, 38.2; HCOSEY. To a solution of the diester of **2** (0.1 g, 0.2 mmol) in MeOH (5 mL), THF (5 mL) and H_2O (5 mL) was added 50 mg of LiOH. The resulting mixture was stirred at rt overnight, acidified to pH 4 (by 5% HCl) and extracted with EtOAc (15 mL x 3). The combined organic layers were condensed and purified by flash chromatography (silica gel; 4:1, EtOAc: MeOH) to afford **2** (80 mg, 86%) as a white solid: ^{1}H NMR (400 MHz, MeOH-d_4): δ 7.89-7.86 (dd, 1H, J = 1.4, 6.4 Hz), 7.51-7.46 (td, 1H, J = 1.7, 7.0 Hz), 7.17 (d, 4H, J = 8.0 Hz), 7.09 (d, 4H, J = 8.0 Hz), 7.04-7.00 (t, 1H, J = 7.2 Hz), 6.95 (d, 1H, J = 8.1 Hz), 4.11 (s, 2H), 3.56 (s, 4H), 3.25 (d, 2H, J = 13.6 Hz), 3.62 (d, 2H, J = 13.6 Hz). ^{13}C NMR (100 MHz, MeOH-d_4): δ 197.3, 176.4, 162.9, 137.6, 136.4, 135.1, 132.3, 130.7, 129.9, 129.0, 123.1, 122.2, 119.3, 73.4, 52.1, 42.7, 39.7.

3-[4-(4-Oxo-4*H*-chromen-2-yl)-phenyl]acrylic acid (3)
A solution of 4'-bromoflavone (0.15 g, 0.5 mmol), palladium (II) acetate (25 mg, 0.02 mmol) and ethyl acrylate (0.27 ml, 2.5 mmol) in 5 ml of anhydrous CH_3CN was sealed in an ace tube (25 mL) under N_2. The mixture was refluxed at 105°C overnight, diluted with EtOAc (30 mL), washed with 1 N HCl (10 mL), saturated $NaHCO_3$ (20 mL) and brine. The combined organic layer was dried over $MgSO_4$, condensed and purified by flash chromatography (silica gel; 10:1 to 1:1, hexane: EtOAc) to afford 3-[4-(4-oxo-4-H-chromen-2-yl)-phenyl]-acrylic acid ethyl ester (0.13g; yield: 97%) as a pale yellow solid: mp 172-173°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.13-8.16 (m, 1 H), 7.87 (d, 2H, J = 8.5 Hz), 7.57-7.65 (m, 4 H), 7.49 (d, 1H, J = 8.4 Hz), 7.33-7.37 (t, 1H, J = 8.1 Hz), 6.77 (s, 1 H), 6.46 (d, 1H, J = 16.0 Hz), 4.19-4.24 (q, 2H, J = 7.1 Hz), 1.28 (t, 3H, J = 7.1 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 178.6, 166.9, 162.7, 156.6, 143.3, 137.9, 134.3, 133.4, 128.9, 127.1, 126.1, 125.7, 124.4, 120.8, 118.4, 108.3, 61.1, 23.3. A solution of 3-[4-(4-oxo-4-H-chromen-2-yl)-phenyl]-acrylic acid ethyl ester (80 mg, 0.2 mmol) in MeOH: THF: H_2O (1:1:1) (15 mL) was added 15 mg of LiOH and stirred at rt for 1 hr. The resulting mixture was neutralized to pH = 4.0 and then condensed and purified by flash chromatography (silica gel; 1:1:0.01, hexane: EtOAc: HCOOH; 40%) to afford compound **3** (25 mg; yield: 34%) as a yellow solid: ^{1}H NMR (400 MHz, MeOH-d_4): δ 8.09 (d, 1H, J = 7.9 Hz), 7.96 (d, 2H, J = 8.5 Hz), 7.60-7.64 (m, 6 H), 7.52 (m, 1H), 7.42 (t, 1H, J = 8.0), 6.87 (s, 1H), 6.52 (d, 1H, J = 16.0 Hz).

4-[(4-Oxo-4*H*-chromen-2-yl)carbonylamino]benzoic acid (4)

To the solution of chromone-2-carboxylic acid (2g, 10.518 mmol) in CH_2Cl_2, was added DMAP (0.963g, 7.885 mmol) and methyl-4-aminobenzoate (1.191g, 7.885 mmol). DMSO was added to completely dissolve the reactants. Using a syringe, DCC (7.885ml, 7.885 mmol) was added drop–wise to the reaction mixture with stirring. The reaction was carried out under N_2 and was stirred for 48 h. The reaction mixture was filtered, cooled in an ice bath and filtered again. Solvent was evaporated, CH_2Cl_2 added and the mixture was washed with water, a 1N HCl solution, water, a 10% NaOH solution, and water. The organic phase was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The product was purified by column chromatography using EtOAc: hexane (5:1) methyl 4-{(4-oxo-4H-chromen-2-yl)carbonylamino}benzoate was isolated as a pure white solid in 67% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.6 (s, 1H), 8.25 (d, 1H, Ar, J = 6.9 Hz), 8.1 (d, 2H, Ar, J = 7.2 Hz), 7.84–7.77 (m, 3H, Ar), 7.61 (d, 1H, Ar, J = 8.1 Hz), 7.51 (m, 1H, Ar), 7.29 (s, 1H, Ar), 3.93 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 178.14, 166.65, 157.47, 155.48, 154.40, 140.71, 135.16, 131.40, 127.52, 126.70, 124.76, 120.07, 118.35, 113.37, 52.53, 0.34. The methyl 4-{(4-oxo-4H-chromen-2-yl)carbonylamino}benzoate (0.610g, 1.887 mmol) was dissolved in MeOH/ H_2O/CH_2Cl_2. LiOH (0.136g, 5.664 mmol) was added to the solution and the reaction was stirred at rt under nitrogen for 2 – 7 h. When TLC indicated the reaction was complete the mixture was extracted with CH_2Cl_2. The aqueous phase was then made acidic by dropwise addition of HCl and extracted with EtOAc (50 mL x 3). The organic phase was dried over $MgSO_4$, evaporated under educed pressure and purified by column chromatography using EtOAc: MeOH: HCOOH (5:1:0.1) to afford 4 in 7% yield. ^{1}H NMR (500 MHz, MeOH-d_4,) δ 11.0 (s, 1H), 8.25 (d, 1H, Ar), 8.15 (s, 1H, Ar), 7.9 (d, 2H, Ar), 7.7 (m, 1H, Ar), 7.5 (m, 1H, Ar), 6.9 (d, 2H, Ar), 6.7 (s, 1H, Ar), 6.6 (s, 1H, Ar).

4-[(4-Oxo-4*H*-chromen-3-yl)carbonylamino]benzoic acid (5)

To the solution of chromone-3-carboxylic acid (1g, 5.259 mmol) in CH_2Cl_2, was added DMAP (0.963g, 7.885 mmol) and methyl-4-aminobenzoate (1.191g, 7.885 mmol). DMSO was added to completely dissolve the reactants. Using a syringe, DCC (7.885mL, 7.885 mmol) was added drop–wise to the reaction mixture with stirring. The reaction was stirred for 48 h under nitrogen. The reaction was filtered and the filtrate chilled in an ice bath and filtered again. Solvent was evaporated and the residue dissolved in CH_2Cl_2. The organic phase was washed with water, 1 N HCl, water, 10% NaOH, and water. The organic phase was dried over $MgSO_4$, filtered, and concentrated under reduced pressure to provide an oil that was purified by column chromatography using EtOAc: hexane (1:22) and recrystallized from EtOAc/hexane to afford methyl ester as yellow needle-shaped crystals (79% yield). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.99–7.97 (d, 2H, J = 6.8 Hz), 7.64 (q, 1H, J = 1.6 Hz, J = 6.5 Hz), 7.52 (q, 1H, J = 4.2 Hz, J = 8.1 Hz), 7.35 (m, 1H), 7.07 (d, 2H, J = 8.8 Hz), 6.9 (q, 1H, J = 1.04 Hz, J = 7.3 Hz), 6.8 (m, 1H), 6.04 (d, 1H, J = 8.2 Hz), 3.84 (s, 3H). The methyl 4-{(4-oxo-4H-chromen-3-yl)carbonylamino}benzoate (0.610g, 1.887 mmol,) and LiOH (0.136g, 5.664 mmol) were dissolved in MeOH:H_2O:CH_2Cl_2 in 1:1:2 ratio. The reaction mixture was stirred at rt under nitrogen for 2 – 7 h and monitored by TLC using CH_2Cl_2: EtOAc (2:1). The reaction was worked up by extracting the mixture with CH_2Cl_2. The solvent was evaporated in vacuum. Water was added and made acidic by concentrated HCl to pH 2 – 3. The aqueous layer was extracted with EtOAc (50 mL x 3) dried over $MgSO_4$ and concentrated. The product was cleaned by column chromatography using EtOAc: MeOH: HCOOH (5:1:0.1) to afford **5** in 17% yield. ^{1}H NMR (400 MHz, DMSO–d_6) δ 13.77 (s, 1H), 8.51 (t, 1H, Ar, J = 12 Hz), 8.18 (q, 1H, Ar, J = 8.5 Hz, J = 4.2 Hz), 8.10–8.06 (m, 2H, Ar), 7.97–7.91 (m, 1H, Ar), 7.65–7.60 (m, 1H), 7.46 (d, 1H, Ar, J = 8.7 Hz), 7.11–7.05 (m, 1H, Ar), 6.79 (d, 1H, Ar, J = 8.4 Hz), 6.45 (d, 1H, Ar, J = 8.3 Hz).

4-[(4-Oxo-4*H*-chromen-3-yl)carbonylamino]benzyl alcohol (6)

To a solution of chromone-3-carboxylic acid (1 g, 5.259 mmol) in anhydrous DMF, was added DMAP (0.1927 g, 1.577 mmol) and 4-aminobenzyl alcohol (0.648 g, 5.259 mmol) under nitrogen. DCC (5.259 mL, 5.259 mmol) was added drop–wise to the mixture via syringe and the reaction was stirred for 48 h and monitored by TLC using hexane: EtOAc (5:1). When TLC indicated no change in the reaction, it was filtered and the filtrate chilled in an ice bath and filtered again. Solvent was evaporated, CH_2Cl_2 was added and the organic phase washed with water, 1 N HCl, water, 10% NaOH, and water. The organic phase was dried over $MgSO_4$, filtered, concentrated and the residue chromatographed using EtOAc: hexane (1:20) to yield (58%) the product 6. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (dd, 1H, Ar, J = 1.2 Hz, J = 5.2 Hz), 7.54 (dd, 1H, Ar, J = 6.4 Hz, J = 3.6 Hz), 7.39–7.36 (m, 2H, Ar), 7.12 (dd, 2H, Ar, J = 1.6 Hz, J = 6.7 Hz), 6.96 (dd, 1H, Ar, J = 1.0 Hz, J = 7.2 Hz), 6.85 (t, 1H, Ar, J = 1.0 Hz), 6.03 (d, 1H, Ar, J = 8 Hz), 4.68 (s, 2H) 3.18 (s, 1H).

4-[(4-Oxo-4*H*-chromen-2-yl)carbonylamino]benzyl alcohol (7)

To a solution of chromone-2-carboxylic acid (1 g, 5.259 mmol) in anhydrous DMF, was added DMAP (0.1927 g, 1.577 mmol) and 4-aminobenzyl alcohol (0.648 g, 5.259 mmol) under a nitrogen atmosphere. Using a syringe, DCC (5.259 mL, 5.259 mmol of 1M solution in CH_2Cl_2) was added drop-wise, stirred for 48 h, and monitored by TLC using hexane: EtOAc (5:1). The reaction was filtered, the filtrate chilled and filtered again. Solvent was evaporated, CH_2Cl_2 was added

and the organic phase washed with water, 1 N HCl, water, 10% NaOH, and water. The organic phase was dried over MgSO4, filtered, concentrated in vacuo, and the residue purified by column chromatography (EtOAc: hexane; 1:20) to afford the product 7 in moderate yield (45%).^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.03 (d, 1H, Ar, J = 7.8 Hz), 7.78–7.70 (m, 2H), 7.64 (d, 2H, Ar, J = 8.4 Hz), 7.41 (q, 1H, Ar, J = 6.8 Hz, J = 1.1 Hz), 7.27 (d, 2H, Ar, J = 8.4 Hz), 6.95 (s, 1H, Ar), 4.49 (s, 2H), 3.2 (s, 1H).

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