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Design and development of FGF-23 antagonists: Definition of the pharmacophore and initial structure-activity relationships probed by synthetic analogues

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ABSTRACT

Hereditary hypophosphatemic disorders, TIO, and CKD conditions are believed to be influenced by an excess of Fibroblast Growth Factor-23 (FGF-23) which activates a binary renal FGFRs / α-Klotho complex to regulate homeostatic metabolism of phosphate and vitamin D. Adaptive FGF-23 responses from CKD patients with excess FGF-23 frequently lead to increased mortality from cardiovascular disease. A reversibly binding small molecule therapeutic has yet to emerge from research and development in this area. Current outcomes described in this work highlight efforts related to lead identification and modification using organic synthesis of strategic analogues to probe structure-activity relationships and preliminarily define the pharmacophore of a computationally derived hit obtained from virtual high-throughput screening. Synthetic strategies for the initial hit and analogue preparation, as well as preliminary cellular *in vitro* assay results highlighting sub micromolar inhibition of the FGF-23 signaling sequence at a concentration well below cytotoxicity are reported herein.

1. Introduction

FGF-23 is a bone-derived signaling biomolecule that activates a FGFR/α-Klotho binary complex in the kidney to regulate renal metabolism of phosphate and vitamin-D.[1] Excess FGF-23 results in rare hereditary hypophosphatemic disorders, such as X-linked hypophosphatemia[2] (XLH), the autosomal recessive hypophoshatemic (ARH) bone-softening disorder ricketts,[3] and acquired tumor-induced osteomalacia (TIO).[4] Adaptive increases in FGF-23 also maintain phosphate and vitamin D homeostasis in chronic kidney disease (CKD) and is associated with increased cardiovascular (CV) mortality.[5] Recently, antagonizing FGF-23 with a blocking antibody has been successful in treating hypophosphatemic disorders caused by excess FGF-23. In this regard, a FGF-23 monoclonal antibody KRN23 (Crysvita®, burosumab) has been approved for treatment of XLH.[6] There is an unmet clinical therapeutic need for an efficacious small molecule antagonist of FGF-23 that is selective, reversible, and non-biologic in nature as over suppression of FGF-23 has potential toxicities, including hyperphosphatemia and vascular calcifications. Because of the disadvantages associated with parenteral administration of biologics, developing an orally available small molecule drug to block the FGF-23 signaling sequence would have several potential advantages, including ease of administration, shorter half-life, effective dose-titration to more precisely inhibit FGF-23, and possibly result in greater efficacy and safety.

Virtual high-throughput screening (*v*HTs) via supercomputing utilizing structure-based molecular dynamics simulations[7] employing ensemble docking strategies[8] afforded ZINC13407541 (parent molecule, named as **1**) as an *in silico* hit for selective FGF-23 antagonism (Fig. 1). Cellular assays validated **1** as a selective inhibitor of the FGF-23 signaling sequence in an *in vitro* heterologous cell expression model, as well as isolated renal tubules *ex vivo*.[9] Preliminary animal model studies with a murine species that overexpressed FGF-23 resulted in dose-dependent inhibition, as well as partial reversal of hypophoshatemic effects. With *in vitro* and preliminary animal model efficacy of **1** confirmed, medicinal chemistry efforts transitioned to synthetic route optimization and elaboration towards formulating a lead compound from the original hit while further refining the original scaffold for greater efficacy and ease of synthetic preparation. Dissemination of

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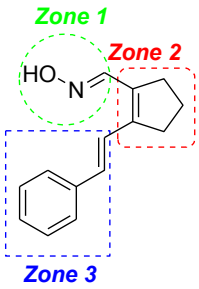


Fig. 1. Original *v*HTS hit ZINC13407541 (1) and focus areas for analogue development.

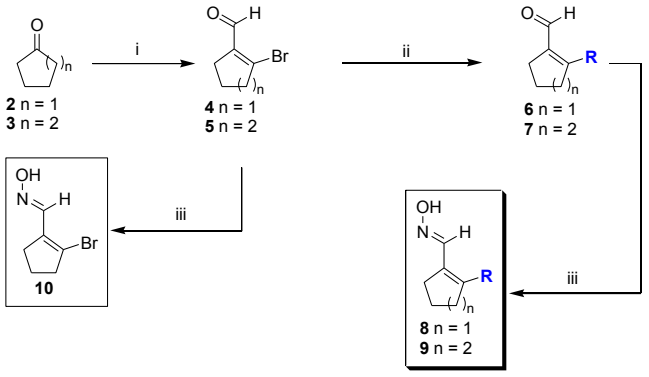
the synthetic route to the prepared antagonist analogues, definition of the pharmacophore, probing of structure–activity relationships via synthetic analogues, and the optimization of *in vitro* potency of prepared molecules are reported.

2. Results

2.1. Synthesis

The synthetic construction of analogues as novel FGF-23 antagonists discovered from *v*HTS affording **1** was anchored from a common scaffold derived in several synthetic steps from readily available cycloalkanone starting materials (Scheme 1). Retrosynthetic analysis of **1** using standard functional group interconversions of oxime formation[10] and metal-mediated transformations through the Suzuki-Miyaura cross-coupling[11] via known chemistry was envisioned.[12] The convergency of the route allowed maximum flexibility of functionality towards pharmacophore mapping and optimization towards lead identification. Analogues were proposed to initially focus on variation of functionality to maintain conjugation of the extended π-system through the carbonyl and cycloalkene. Evaluation of ring size through the 5- and 6-membered common rings, in addition to heteroarene constructs was also performed (Scheme 2).

arene and heteroarene constructs were evaluated. Extension of this strategy towards aryl and Conversion of cyclopentanone to 2-bromocyclopentene carbaldehyde using a modified Vilsmeier[13] protocol adapted from Lipton[14] afforded the desired synthon for metal-mediated coupling (**4**) in 70% yield. This reaction was amenable towards the formation of the 5-membered bromo-cyclopentene carbaldehyde **4** or the 6-membered homologue **5**.[15] Moving forward, treatment of **4** or **5** with potassium styrenyltrifluoroborate[16] using Pd-catalysis via Suzuki-Miyaura cross-coupling[17] conditions optimized in our laboratory[18]



Scheme 1. Synthesis of compounds 2-10. Reagents and conditions: (i) PBr_3, DMF, rt; (ii) $Pd(OAc)_2$ (5 mol%), RuPhos (10 mol%), RBR_n (1.2 equiv), Cs_2CO_3 (3 equiv), Tol:H_2O (4:1), 110 °C, 16 h; (iii) $NH_2OH{\cdot}HCl$, NaOAc, EtOH / H_2O, rt.

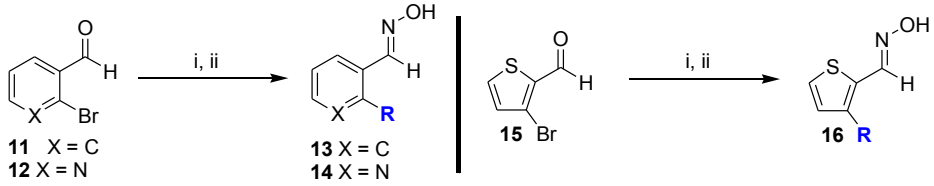
resulted in the production of the necessary advanced aldehyde intermediates **6** and **7** for subsequent functional group interconversion.[19] Conversion to the oxime **1** was successful under standard conditions resulting in a 46% yield over three steps from cyclopentanone (**2**) on micro scale. During route scouting and validation it was observed that **4** could be directly converted into the oxime **10** in high purity.[20] Intermediate **10** was evaluated in parallel Suzuki-Miyaura cross-coupling reactions towards the production of the functionalized oxime with potassium styrenyltrifluoroborate, but did not afford the desired product in comparable yield, or purity. The chemistry described in Scheme 1 was the basis for the production of all requisite functionalized aldehydes, including the pyridine, benzene, and thiophene oxime analogues of **1** (Scheme 2) [21]

With an established protocol to install the requisite carbon functionality onto the desired scaffolds, focus turned towards the completion of the oxime derivatives described in Table 1 for the definition of the pharmacophore and evaluation of structure-activity relationships probed by synthetic analogues. The original *v*HTS hit was divided into three zones for functional group manipulation towards understanding the impacts of various structural features of a given antagonist analogue on *in vitro* efficacy (Fig. 1). Zone 1 analogues evaluated the significance of the hydrogen bond donor oxime and related methyl oxime derivative. Zone 2 analogues studied the impact of saturated ring size through the common 5- and 6-membered cycloalkanes, in addition to substitution of phenyl, pyridinyl, and thiophene cores. Whereas, Zone 3 analogues of **1** probed the necessity of extended π-conjugation through the styrenyl, or direct connect aryl derivatives. The initial optimization analogue scope of **1** encompassed diverse functionality including conjugated styrenyl derivatives with aldehydes **4**, **5**, **11**, **12**, and **15**. Aliphatic analogues (**8f**, **8g**, and **8t**) were critical for evaluating the importance of conjugation to maintenance of more potent biological activity. A vinyl derivative lacking any aryl ring (**8u**), as well as direct connect aryl moieties (**8l**-**8s**) were prepared. Aryl moieties with broad substitution patterns (*o*, *m*, *p*, or poly-) were explored while concomitantly ascertaining the significance of resonance- and electron-donating, in addition to electron-withdrawing functionality. Most oxime end products were afforded as one major stereoisomer in above 75% average isolated, purified yield. Structural confirmation of prepared analogues was facilitated via ^{1}H- and ^{13}C NMR in concert with high-resolution mass spectrometry. The unoptimized current synthetic route is reproducibly robust and can support future assay studies without extensive reoptimization.[22] With the realization of a viable synthetic strategy to produce relevant analogues of **1** for structure–activity studies, the focus of the research shifted to evaluating the *in vitro* efficacy of the prepared analogues.

2.2. In vitro efficacy

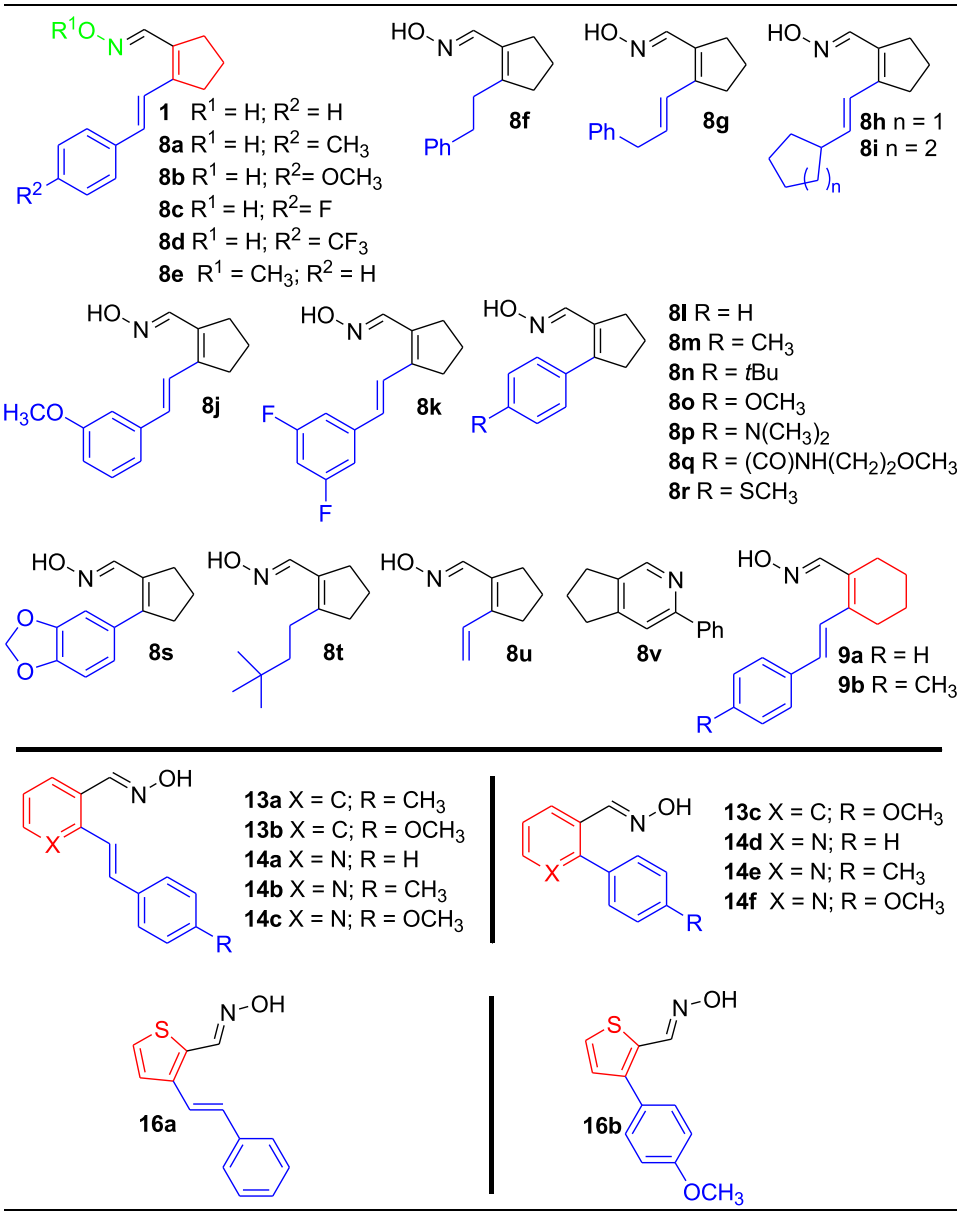
To evaluate the potency of the newly synthesized compounds, *in vitro* efficacy of the prepared antagonists, as measured by percent inhibition using HEK-293 T cells expressing FGFRs/α-Klotho and FGF-23-induced ERK reporter activation as the read out, were performed (Fig. 2). Five distinct groups at 10 μM concentration were identified: Group I = 100% inhibition, Group II = 70–90% inhibition, Group III = 50–70% inhibition, Group IV = 20–50% inhibition, and Group V < 20% inhibition. Further structure / activity analysis revealed that the aldehyde precursor to **1**[23] (**6a**) demonstrated 6-fold lower biological activity (%Max inhibition 46%) than **1** which underscores the significance of the oxime moiety to the pharmacophore of **1** in Group IV. The potential for hydrogen bonding of the oxime versus the methyl oxime **8e** which, displayed <20% inhibition, signifies the importance of this functional group to the antagonist pharmacophore. Removal of aromatic functionality (**8h**, **8i**, or **8t**), or manipulation of the conjugation in **1** with analogues **8f** and **8g** proved deleterious to biological activity.

Assessment of substitution of weakly donating alkyl substitutents on the aryl ring of **8a** of core **4**, as well as the resonance-donating methoxy-substituent of **8b** highlighted the significance of these structural



Scheme 2. Synthesis of compounds 11–14. Reagents and conditions: (i) $Pd(OAc)_2$ (5 mol%), RuPhos (10 mol%), RBR_n (1.2 equiv), Cs_2CO_3 (3 equiv), Tol:H_2O (4:1), 110 °C, 16 h; (ii) NH_2OH·HCl, NaOAc, EtOH / H_2O, rt, 16 h.

Table 1
FGF-23 antagonists prepared based on the structure of the original hit.



amendments to overall potency. Interestingly, manipulation of the cores from **4** to **5**, **11**, or **12**, while retaining the 4-methylstyrenyl substituent (**9b**, **13a**, or **14b**) as part of the Zone 2 analogues, afforded the most potent analogues to **1**, while demonstrating the relevance of this substituent to biological activity. Electrocyclization analogue **8v**, afforded during chromatography of **1**, and postulated to potentially be an *in vivo* metabolite of **1**, produced poor activity results as a constituent of Group V did not validate the aforementioned hypothesis.[24] Subsequent effort targeted the preparation of analogues which were devoid of the trans-alkenyl double bond which bridged the core to the aryl substituents. Excision of the interstitial trans-double bond alleviates two rotatable bonds,[25] modulates lipophilicity slightly, and affords more rapid access to future functionally group diverse examples *vide infra*. Compounds **8m** and **8o**, direct-connect, aromatic derivatives of **8a** and **8b**, which negated the vinyl group, demonstrated lower than half biological activity compared to **1** and further validated the relevance of extension of

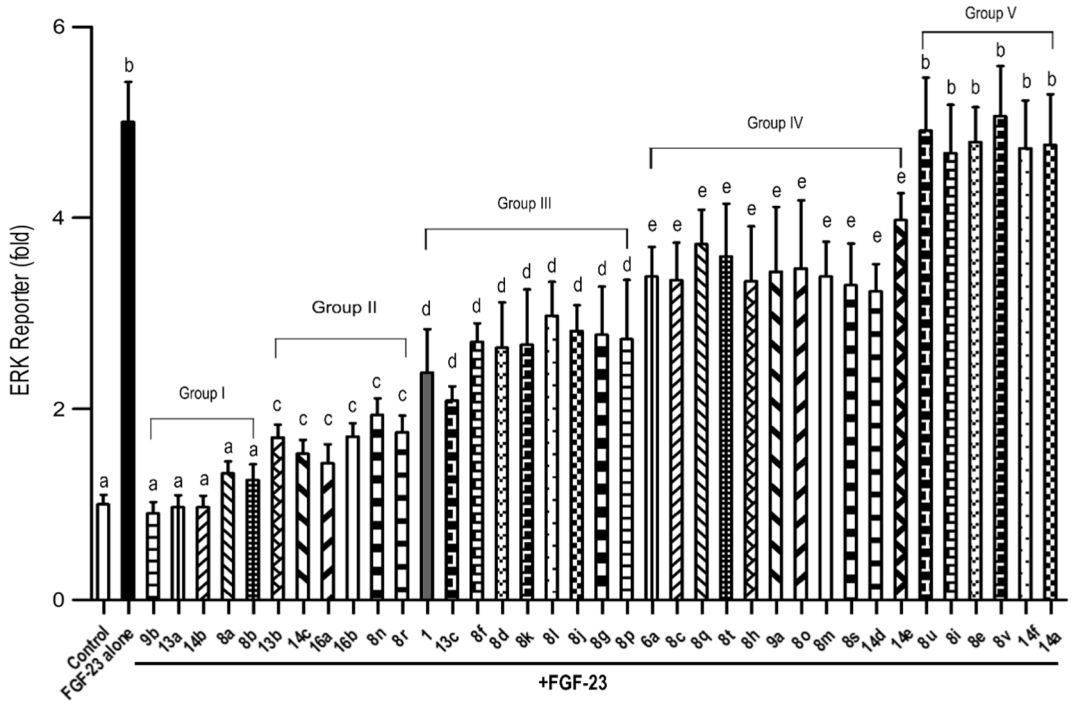


Fig. 2. Comparison of analogues of 1 based on ERK reporter activities at the concentration of 10 μM. Values (mean ± SEM, n = 3–5) with different superscripts (a-e) are significantly different at $P < 0.05$.

conjugation, or electron density, as potential influences on increased activity. Thiophene analogues of **1** were extremely challenging to synthesize and purify. Substantive efforts afforded **16a** and **16b** which performed inferiorly to **1**. Probing the structural nature of the oxime with respect to biological activity as part of the Zone 3 analogues, including the methyl oxime **8a**, aldoxime **1** afforded slightly higher potency. Based on the initial *in vitro* results presented, eight analogues were selected from Groups I to IV for IC_{50} determinations (Table 2).

The IC_{50} values of prepared FGF-23 antagonist analogues evaluated ranged from 0.14 μM (**13a**) to 31 μM (**6a**) with a ranked order of **13a** (0.14 μM), **8a** (0.20 μM), **8c** (0.37 μM), **14b** (0.39 μM), **9b** (0.52 μM), **8n** (2.79 μM), **8 l** (10 μM), **8o** (12.3 μM) and **6a** (31 μM), respectively. **8n, 8 l,** and **8o** decreased maximum inhibition activity (%Max inhibition 60 ~ 70%) in Group II, III, and IV. On the aryl ring of **8a** and **8c**, a 4-position electron-donating substituent CH_3 for **8a** and the resonance-donating substituent OCH_3 for **8c** largely increases the efficacy, leading to 10 ~ 25-fold higher potency for inhibiting FGF-23 activity when compared to **1** (IC_{50} 5.0 μM) in Group I. The presence of the double bond in **8c** compared to **8o** resulted in an order of magnitude increase in activity for the former, as compared to the latter. In addition, incorporation of a 6-membered cyclohexenyl ring (**9b**), aromatic ring (**13a**) or heteroaromatic core (**14b**) on these analogues resulted in 10 ~ 36-fold higher potency for inhibiting FGF-23 activity as opposed to **1** (IC_{50} 5.0 μM) in Group I. Cytotoxicity assays revealed that all the test compounds have no obvious cytotoxicity from 10^{-9} M to 10^{-5} M, which is further underscored by the fact that IC_{50} values were dramatically lower than EC_{50} values. Only **8n** showed markedly stimulated cellular LDH release at a concentration of 10^{-4} M with 2.30×10^2 μM EC_{50}. The EC_{50} values of other compounds with a ranked order are **8a** (5.70×10^2 μM), **13a** (6.90×10^2 μM), 1 (1.41×10^3 μM), **8o** (1.91×10^3 μM), and **14b** (2.41 $\times$ 10^3 μM), respectively. Future studies will examine the adsorption, distribution, metabolism, excretion, selectivity and toxicity of these lead compounds.

Table 2
Determination of efficacy (IC_{50}) and cytotoxicity (EC_{50}) for select potent analogues.

Compound	IC50 (μM)	EC50 (μM)
1	5.0	1.41×10^3
13a	0.14	6.90×10^2
8a	0.20	5.70×10^2
8c	0.37	–
14b	0.39	2.41×10^3
9b	0.52	–
8n	2.79	2.30×10^2
8l	10.0	–
8o	12.3	1.91×10^3

3. Conclusion

In conclusion, we have described an efficiently convergent and diversified approach to small-molecule antagonists of the bone-derived signaling biomolecule FGF-23 from an initial *v*HTS hit. Structure-activity relationships were probed from the preparation of expansive synthetic analogues which have preliminary defined the requisite pharmacophore of this antagonist class requiring a polar oxime with a conjugated aryl group with electron-donating substituents being able to achieve sub 1 μM inhibition in the FGF-23 signaling sequence from *in vitro*, cellular IC_{50} assays. Subsequent lead optimization efforts with respect to first pass metabolic stability, oral availability, toxicity, and animal model studies are ongoing in these laboratories and will be reported in due course.

4. Experimental section

General Considerations: All reagents were purchased from U.S. chemical suppliers, stored according to published protocols, and used as received unless indicated otherwise. All experiments were performed in oven- or flame-dried glassware. Reaction progress was monitored using thin-layer chromatography on glass-backed silica gel plates and/or ^1H NMR analysis of crude reaction mixtures. R_F values for compounds that resulted in a concentrically observed spot on normal phase silica gel are reported using the conditions listed. All melting points are reported as observed and uncorrected. All reported yields listed are for pure

compounds and corrected for residual solvent or stereoisomeric impurities, if applicable, from ^{1}H NMR spectroscopy unless otherwise indicated. All ^{1}H and ^{13}C NMR data was acquired from a 500 MHz multinuclear spectrometer with broad-band N_2 cryoprobe. Chemical shifts are reported using the δ scale and are referenced to the residual solvent signal: $CDCl_3$ (δ 7.26) and CD_3OD (3.31) for ^{1}H NMR and chloroform (δ 77.16), CD_3OD (39.00), and $(CD_3)_2CO$ (29.84) for ^{13}C NMR. Splittings are reported as follows: (s) = singlet, (d) = doublet, (t) = triplet, (dd) = doublet of doublets, (ddd) = doublet of doublet of doublets, (dt) = doublet of triplets, (br) = broad, (m) = multiplet, and pent = pentet. Infrared spectral data was acquired from the (form) listed. High resolution mass spectrometry (HRMS) data was obtained utilizing electron impact ionization (EI) with a magnetic sector (EBE trisector), double focusing-geometry mass analyzer. The compounds were stored in the −20 °C freezer and were tested via *in vitro* assay. Recombinant human FGF-23 was purchased from R&D Systems (Minneapolis, MN, USA).

General Procedure for Oxime Formation: To an 8 mL reaction vial equipped with a magnetic stir bar at ambient temperature was charged the required aldehyde, sodium acetate (1.50 equiv), and hydroxylamine hydrochloride (1.50 equiv) in ethanol:H_2O (3:1) (10 vol) at ambient temperature. The reaction was continued for 16 h upon which time an aliquot was removed and analyzed by ^{1}H NMR. Concentration of the crude reaction mixtures under reduced pressure at ambient temperature followed by purification on normal phase silica gel using automated flash-column chromatography with MTBE:hexanes, EtOAc:hexanes, or MeOH:DCM gradient mobile phases afforded the compounds described in the listed yields.

2-Styryl-cyclopent-1-enecarbaldehyde oxime (1): Prepared according to the general procedure discussed above with **6a**[26] (0.54 mmol, 1.00 equiv) and hydroxylamine hydrochloride, R_F = 0.58, 20% MTBE:hexanes; purified using automated flash column chromatography using an MTBE:hexanes gradient mobile phase employing a 5% isocratic hold; isolated yield 0.195 g, 93%; orange solid; mp = 149.7–152.9 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.41 (s, 1H), 7.45 (d, J = 7.5 Hz, 2H), 7.36–7.32 (m, 2H), 7.28–7.24 (m, 1H), 7.23 (d, J = 16.0 Hz, 1H), 6.62 (d, J = 16.0 Hz, 1H), 2.78–2.69 (m, 4H), 1.98 (pent, J = 7.5 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 146.4, 145.8, 137.2, 133.5, 132.2, 128.9, 128.2, 126.8, 121.2, 33.8, 32.9, 21.9; IR (ATR-$CDCl_3$): $\overline{\upsilon}_{max}$ = 3247, 3032, 2953, 2850, 1649, 1599, 1510, 1006, 948, 939, 753, 693 cm^{-1}; HRMS (EI): *m/z* calculated for $C_{14}H_{15}NO$: 213.1154; found: 213.1155.

2-(2-*p*-Tolyl-vinyl)-cyclopent-1-enecarbaldehyde oxime (8a): Prepared according to the general procedure discussed above with **6b** (0.30 mmol, 1.00 equiv) and hydroxylamine hydrochloride, R_F = 0.51, 20% MTBE:hexanes; purified using automated flash column chromatography using an MTBE:hexanes gradient mobile phase employing a 5% isocratic hold; isolated yield 0.051 g, 75%; off-white solid; mp = 172.9–177.4 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.40 (s, 1H), 7.35 (d, J = 7.5 Hz, 2H), 7.19 (d, J = 16.0 Hz, 1H), 7.15 (d, J = 7.5 Hz, 2H), 6.60 (d, J = 16.0 Hz, 1H), 2.78–2.68 (m, 4H), 2.35 (s, 3H), 1.97 (pent, J = 7.5 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 146.4, 145.6, 138.0, 134.4, 132.9, 132.0, 129.5, 126.6, 120.2, 33.7, 32.7, 21.7, 21.2; IR (ATR-$CDCl_3$): $\overline{\upsilon}_{max}$ = 3200, 2955, 2847, 1615, 1583, 1511, 1465, 1006, 940, 801 cm^{-1}; HRMS (EI): *m/z* calculated for $C_{15}H_{17}NO$: 227.1310; found: 227.1302.

2-[2-(4-Methoxy-phenyl)-vinyl]-cyclopent-1-enecarbaldehyde oxime (8b): Prepared according to the general procedure discussed above with **6c** (0.44 mmol, 1.00 equiv) and hydroxylamine hydrochloride, R_F = 0.30, 20% EtOAc:hexanes; purified using automated flash column chromatography using an MTBE:hexanes gradient mobile phase; isolated yield 0.074 g, 99%; off-white solid; mp = 151.2–153.0 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.40 (s, 1H), 7.42–7.38 (m, 2H), 7.11 (d, J = 16.0 Hz, 1H), 6.91–6.86 (m, 2H), 6.58 (d, J = 16.0 Hz, 1H), 3.83 (s, 3H), 2.77–2.68 (m, 4H), 1.97 (pent, J = 7.5 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 159.7, 146.5, 14538, 132.4, 131.7, 130.1, 128.1, 119.3, 114.4, 55.5, 33.8, 32.8, 21.8; IR (ATR-$CDCl_3$): $\overline{\upsilon}_{max}$ = 3260, 3032, 3002, 2841, 1602, 1510, 1248, 1174, 904, 819, 726 cm^{-1}; HRMS (EI): *m/z* calculated for $C_{15}H_{17}NO_2$: 243.1259; found: 243.1265.

2-[2-(4-Fluoro-phenyl)-vinyl]-cyclopent-1-enecarbaldehyde oxime (8c): Prepared according to the general procedure discussed above with **6d** (0.30 mmol, 1.00 equiv) and hydroxylamine hydrochloride. The crude mixture was concentrated under reduced pressure and the resulting residue was partitioned between EtOAc:H_2O in a separatory funnel where the organic layer was separated. The aqueous layer was back extracted with 2 × 10 mL portions of ethyl acetate. The combined organic layers were washed with 10 mL of a saturated aqueous NaCl solution, dried over anhydrous sodium sulfate, filtered, and then concentrated under reduced pressure to afford the title compound: R_F = 0.49, 20% MTBE:hexanes; isolated yield 0.067 g, 99%; tan solid; decomposed upon heating for mp analysis; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.40 (s, 1), 7.45–7.40 (m, 2H), 7.14 (d, J = 15.7 Hz, 1H), 7.06–7.01 (m, 2H), 6.58 (d, J = 15.7 Hz, 1H), 2.77 (m, 4H), 1.98 (pent, J = 7.5 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 163.9, 161.7, 146.1, 145.6, 133.5, 133.4 (J = 3.0 Hz), 128.3 (J = 8.5 Hz), 121.0 (J = 2.0 Hz), 115.9 (J = 21.8 Hz), 33.8, 32.9, 21.8; IR (ATR-$CDCl_3$): $\overline{\upsilon}_{max}$ = 3255, 2964, 600, 1507, 1224, 855, 819 cm^{-1}; HRMS (EI): *m/z* calculated for $C_{14}H_{14}FNO$: 231.1059; found: 231.1062.

2-[2-(4-Trifluoromethyl-phenyl)-vinyl]-cyclopent-1-enecarbaldehyde oxime (8d): Prepared according to the general procedure discussed above with **6e** (0.86 mmol, 1.00 equiv) and hydroxylamine hydrochloride. The crude mixture was concentrated under reduced pressure and the resulting residue was partitioned between EtOAc:H_2O in a separatory funnel where the organic layer was separated. The aqueous layer was back extracted with 2 × 10 mL portions of ethyl acetate. The combined organic layers were washed with 10 mL of a saturated aqueous NaCl solution, dried over anhydrous sodium sulfate, filtered, and then concentrated under reduced pressure to afford the title compound, R_F = 0.47, 20% MTBE:hexanes; isolated yield 0.132 g, 52%; gold solid; mp = 179.5–181.2 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.41 (s, 1H), 7.60 (d, J = 8.4 Hz, 2H), 7.55 (d, J = 8.4 Hz, 2H), 7.31 (d, J = 16.0 Hz, 1H), 6.63 (d, J = 16.0 Hz, 1H), 2.80–2.72 (m, 4H), 2.00 (pent, J = 7.5 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 145.9, 144.9, 140.7, 135.3, 130.4, 129.8 (d, J = 31.8 Hz), 126.83, 126.8X (overlaps with 126.83), 125.8 (d, J = 3.6 Hz), 123.5, 33.7, 30.0, 21.8; IR (ATR-$CDCl_3$): $\overline{\upsilon}_{max}$ = 3240, 2965, 2838, 1611, 1457, 1320, 1165, 1119, 1108, 1066, 867, 819 cm^{-1}; HRMS (EI): *m/z* calculated for $C_{15}H_{14}F_3NO$: 281.1027; found: 281.1037.

2-Styryl-cyclopent-1-enecarbaldehyde *O*-methyl-oxime (8e): Prepared according to the general procedure discussed above with **6a** (0.16 mmol, 1.00 equiv) and methoxyamine hydrochloride, R_F = 0.39, 20% MTBE:hexanes; purified using automated flash column chromatography using an MTBE:hexanes gradient mobile phase employing a 5% isocratic hold; isolated yield 0.030 g, 89%; orange solid; mp = 139.0–141.5 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.37 (s, 1H), 7.46–7.42 (m, 2H), 7.36–7.31 (m, 2H), 7.27–7.23 (m, 1H), 7.21 (d, J = 16.0 Hz, 1H), 6.60 (d, J = 16.0 Hz, 1H), 3.94 (s, 3H), 2.74 (t, J = 7.5 Hz, 4H), 1.97 (pent, J = 7.5 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 145.1, 144.8, 137.4, 133.9, 131.8, 128.9, 128.1, 126.7, 121.4, 62.0, 33.8, 33.0, 21.9; IR (ATR-$CDCl_3$): $\overline{\upsilon}_{max}$ = 3031, 2935, 2846, 2816, 1601, 1588, 1495, 1448, 1055, 750, 691 cm^{-1}; HRMS (EI): *m/z* calculated for $C_{15}H_{17}NO$: 227.1310; found: 227.1315.

2-Phenethyl-cyclopent-1-enecarbaldehyde oxime (8f): Prepared according to the general procedure discussed above with **6f** (0.25 mmol, 1.00 equiv) and hydroxylamine hydrochloride, R_F = 0.49, 20% MTBE:hexanes; purified using automated flash column chromatography using an MTBE:hexanes gradient mobile phase employing a 5% isocratic hold; isolated yield 0.020 g, 37%; pale-brown oil; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.99 (s, 1), 6.60–6.53 (m, 2H), 6.51–6.43 (m, 3H), 2.02 (t, J = 7.5 Hz, 1H), 1.88–1.79 (m, 4H), 1.76 (t, J = 7.5 Hz, 2H), 1.15 (pent, J = 7.5 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 149.9, 146.5, 141.5, 130.0, 128.5, 128.4, 126.2, 37.2, 34.7, 32.1, 30.9, 22.0; IR (ATR-$CDCl_3$): $\overline{\upsilon}_{max}$ = 3204, 3062, 2948, 2921, 1640, 1602, 1496, 1453, 968, 927, 745, 703 cm^{-1}; HRMS (EI): *m/z* calculated for $C_{14}H_{17}NO$: 215.1310; found: 215.1305.

2-(3-Phenyl-propenyl)-cyclopent-1-enecarbaldehyde oxime (8g): Prepared according to the general procedure discussed above with **6g** (0.40 mmol, 1.00 equiv) and hydroxylamine hydrochloride: R_F = 0.55, 20% MTBE:hexanes; purified using automated flash column chromatography using an MTBE:hexanes gradient mobile phase employing a 5% isocratic hold; isolated yield 0.060 g, 66%; brown liquid; ^{1}H NMR (500 MHz, $CDCl_3$); *major diastereomer*: δ 8.25 (s, 1H), 7.33–7.27 (m, 2H), 7.24–7.13 (m, 3H), 6.56 (d, J = 15.5 Hz, 1H), 5.93 (dt, J = 15.5, 7.0 Hz, 1H), 3.50 (d, J = 7.0 Hz, 2H), 2.65 (br-t, J = 7.5 Hz, 2H), 2.59 (br-t, J = 7.5 Hz, 2H), 1.89 (pent, J = 7.5 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) *major diastereomer*: δ 146.2, 145.8, 139.9, 133.7, 131.4, 128.8, 128.7, 126.5, 124.2, 39.8, 34.0, 32.6, 21.7; IR (ATR-$CDCl_3$): $\bar{v}_{max}$ = 3526, 3026, 2845, 1602, 1495, 1452, 994, 957, 931, 748, 698 cm^{-1}; HRMS (EI): *m/z* calculated for $C_{15}H_{17}NO$: 227.1310; found: 227.1306.

2-(2-Cyclopentyl-vinyl)-cyclopent-1-enecarbaldehyde oxime (8h): Prepared according to the general procedure discussed above with 2-(2-cyclopentyl-vinyl)-cyclopent-1-enecarbaldehyde (0.34 mmol) and hydroxylamine hydrochloride, R_F = 0.66, 20% MTBE:hexanes; purified using automated flash column chromatography using an MTBE:hexanes gradient mobile phase employing a 5% isocratic hold; isolated yield 0.040 g, 57%; peach solid; mp = 123.4–128.0 °C; ^{1}H NMR (500 MHz, $CDCl_3$) *major oxime diastereomer*: δ 8.26 (s, 1H), 6.49 (d, J = 15.5 Hz, 1H), 5.79 (dd, J = 15.5, 8.0 Hz, 1H), 2.67–2.57 (m, 4H), 2.56–2.49 (m, 1H), 1.90 (pent, J = 7.5 Hz, 2H), 1.86–1.78 (m, 2H), 1.71–1.63 (m, 2H), 1.62–1.55 (m, 2H), 1.37–1.28 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) *major oxime diastereomer*: δ 146.8, 146.5, 140.6, 130.2, 121.3, 44.2, 34.0, 33.4, 32.6, 24.4, 21.8; IR (ATR-$CDCl_3$): $\bar{v}_{max}$ = 3261, 2951, 2868, 1637, 1617, 996, 956, 935 cm^{-1}; HRMS (EI): *m/z* calculated for $C_{13}H_{19}NO$: 205.1467; found: 205.1459.

2-(2-Cyclohexyl-vinyl)-cyclopent-1-enecarbaldehyde oxime (8i): Prepared according to the general procedure discussed above with **6h** (0.49 mmol, 1.00 equiv) and hydroxylamine hydrochloride, R_F = 0.56, 20% EtOAc:hexanes; purified using automated flash column chromatography using an MTBE:hexanes gradient mobile phase; isolated yield 0.060 g, 56%; cream colored solid; mp = 136.2–137.9 °C; ^{1}H NMR (500 MHz, $CDCl_3$) *major diastereomer*: δ 8.27 (s, 1H), 6.47 (d, J = 15.5 Hz, 1H), 5.74 (dd, J = 15.5, 7.5 Hz, 1H), 2.63 (t, J = 7.5 Hz, 2H), 2.59 (t, J = 7.5 Hz, 2H), 1.90 (pent, J = 7.5 Hz, 2H), 1.78–1.71 (m, 4H), 1.70–1.63 (m, 1H), 1.35–1.23 (m, 3H), 1.22–1.06 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) *major diastereomer*: δ 146.7, 146.1, 141.1, 130.6, 120.6, 41.5, 33.9, 33.0, 32.6, 26.2, 26.1, 21.8; IR (ATR-$CDCl_3$): $\bar{v}_{max}$ = 3267, 2994, 2921, 2848, 1637, 1585, 1451, 1003, 955, 933 cm^{-1}; HRMS (EI): *m/z* calculated for $C_{14}H_{21}NO$: 219.1623; found: 219.1627.

2-[2-(3-Methoxy-phenyl)-vinyl]-cyclopent-1-enecarbaldehyde oxime (8j): Prepared according to the general procedure discussed above with **6i** (0.35 mmol, 1.00 equiv) and hydroxylamine hydrochloride, R_F = 0.41, 20% MTBE:hexanes; purified using automated flash column chromatography using an MTBE:hexanes gradient mobile phase employing a 5% isocratic hold; isolated yield 0.040 g, 51%; off-white solid; mp = 156.4–158.8 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.41 (s, 1H), 7.28–7.24 (m, 1H), 7.22 (d, J = 16.0 Hz, 1H), 7.07–7.03 (m, 1H), 6.99–6.97 (m, 1H), 6.82 (ddd, J = 8.2, 2.4, 0.8 Hz), 6.58 (d, J = 16.0 Hz, 1H), 3.85 (s, 3H), 2.78–2.68 (m, 4H), 1.98 (pent, J = 7.5 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 160.1, 146.6, 145.4, 138.7, 133.8, 132.0, 129.9, 121.5, 119.6, 114.0, 111.8, 55.4, 33.8, 32.9, 21.8; IR (ATR-$CDCl_3$): $\bar{v}_{max}$ = 3164, 3002, 2837, 1603, 1575, 1490, 1433, 1261, 1044, 950, 770, 684 cm^{-1}; HRMS (EI): *m/z* calculated for $C_{15}H_{17}NO_2$: 234.1259; found: 243.1258.

2-[2-(3,5-Difluoro-phenyl)-vinyl]-cyclopent-1-enecarbaldehyde oxime (8k): Prepared according to the general procedure discussed above with **6j** (0.30 mmol, 1.00 equiv) and hydroxylamine hydrochloride, R_F = 0.55, 20% MTBE:hexanes; purified using automated flash column chromatography using an MTBE:hexanes gradient mobile phase employing a 5% isocratic hold; isolated yield 0.066 g, 88%; pale-pink solid; mp = 171.4–176.1 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.38 (s, 1H), 7.43 (br-s, 1H), 7.20 (d, J = 16.0 Hz, 1H), 6.97–6.92 (m, 2H), 6.72–6.67 (m, 1H), 6.50 (d, J = 16.0 Hz, 1H), 2.77–2.69 (m, 4H), 1.99 (pent, J = 7.5 Hz, 2H); ^{13}C NMR (125 MHz, $(CD_3)_2CO$): δ 164.1 (dd, J = 250.0, 13.0 Hz), 145.8, 143.1, 142.6, 138.0, 129.6 (t, J = 3.0 Hz), 125.4, 110.2 (dd, J = 19.5, 5.5 Hz), 103.1 (t, J = 26.5 Hz), 33.9, 33.7, 23.3; IR (ATR-$CDCl_3$): $\bar{v}_{max}$ = 3181, 3078, 2921, 2851, 1612, 1590, 1437, 1122, 980, 951 cm^{-1}; HRMS (EI): *m/z* calculated for $C_{14}H_{13}F_2NO$: 249.0965; found: 249.0970.

2-Phenyl-cyclopent-1-enecarbaldehyde oxime (8l): Prepared according to the general procedure discussed above with **6k** (0.86 mmol, 1.00 equiv) and hydroxylamine hydrochloride, R_F = 0.34, 10% MTBE:hexanes; purified using automated flash column chromatography using an MTBE:hexanes gradient mobile phase employing a 5% isocratic hold; isolated yield 0.118 g, 74%; yellow solid; mp = 105.1–107.1 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.10 (s, 1H), 7.39–7.35 (m, 2H), 7.32–7.27 (m, 3H), 2.90–2.84 (m, 2H), 2.79–2.74 (m, 2H), 2.02 (pent, J = 7.5 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 148.6, 148.2, 136.7, 131.8, 128.4, 128.0, 127.8, 38.4, 33.0, 22.0; IR (ATR-$CDCl_3$): $\bar{v}_{max}$ = 3261, 3055, 3953, 3849, 1601, 1620, 1493, 967, 760, 698 cm^{-1}; HRMS (EI): *m/z* calculated for $C_{12}H_{13}NO$: 187.0997; found: 187.1000.

2-*p*-Tolyl-cyclopent-1-enecarbaldehyde oxime (8m): Prepared according to the general procedure discussed above with **6l** (0.46 mmol, 1.00 equiv) and hydroxylamine hydrochloride, R_F = 0.39, 20% MTBE:hexanes; purified using automated flash column chromatography using an MTBE:hexanes gradient mobile phase employing a 2.5% isocratic hold; isolated yield 0.065 g, 70%; yellow solid; mp = 157.0–159.5 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.20 (s, 1H), 7.34 (s, 4H), 2.96–2.91 (m, 2H), 2.87–2.82 (m, 2H), 2.45 (s, 3H), 2.09 (pent, J = 7.5 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 149.2, 148.5, 137.9, 133.8, 130.7, 129.2, 128.1, 38.5, 33.1, 22.1, 21.4; IR (ATR-$CDCl_3$): $\bar{v}_{max}$ = 3529, 2961, 2853, 1620, 1513, 1447, 969, 822 cm^{-1}; HRMS (EI): *m/z* calculated for $C_{13}H_{15}NO$: 201.1154; found: 201.1153.

2-(4-*tert*-Butyl-phenyl)-cyclopent-1-enecarbaldehyde oxime (8n): Prepared according to the general procedure discussed above with **6m** (0.58 mmol, 1.00 equiv) and hydroxylamine hydrochloride, R_F = 0.50, 20% MTBE:hexanes; purified using automated flash column chromatography using an MTBE:hexanes gradient mobile phase employing a 5% isocratic hold; isolated yield 0.060 g, 47%; white solid; mp = 136.0–137.5 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.14 (s, 1H), 7.41–7.37 (m, 2H), 7.25–7.20 (m, 2H), 2.90–2.84 (m, 2H), 2.80–2.73 (m, 2H), 2.01 (pent, J = 7.5 Hz, 2H), 1.35 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 151.1, 148.8, 148.2, 133.8, 130.9, 127.9, 125.4, 39.4, 34.8, 33.1, 31.4, 22.1; IR (ATR-$CDCl_3$): $\bar{v}_{max}$ = 3270, 3036, 2960, 2868, 1617, 1508, 1462, 1442, 969, 834 cm^{-1}; HRMS (EI): *m/z* calculated for $C_{16}H_{21}NO$: 243.1623; found: 243.1620.

2-(4-Methoxy-phenyl)-cyclopent-1-enecarbaldehyde oxime (8o): Prepared according to the general procedure discussed above with **6n** (0.76 mmol, 1.00 equiv) and hydroxylamine hydrochloride, R_F = 0.32, 20% MTBE:hexanes; purified using automated flash column chromatography using an EtOAc:hexanes gradient mobile phase employing a 2.5% isocratic hold; isolated yield 0.085 g, 51%; yellow solid; mp = 103.0–106.0 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.12 (s, 1H), 7.24–7.21 (m, 2H), 6.92–6.88 (m, 2H), 3.83 (s, 3H), 2.86–2.82 (m, 2H), 2.78–2.73 (m, 2H), 2.00 (pent, J = 7.5 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 159.5, 148.5, 148.1, 130.1, 129.4, 129.2, 114.0, 55.4, 38.4, 30.1, 22.0; IR (ATR-$CDCl_3$): $\bar{v}_{max}$ = 3270, 3044, 2838, 1607, 1510, 1462, 1441, 1249, 1178, 1033, 965, 832 cm^{-1}; HRMS (EI): *m/z* calculated for $C_{13}H_{15}NO_2$: 217.1103; found: 217.1098.

2-(4-Dimethylamino-phenyl)-cyclopent-1-enecarbaldehyde oxime (8p): Prepared according to the general procedure discussed above with **6o** (0.35 mmol, 1.00 equiv) and hydroxylamine hydrochloride, R_F = 0.43, 20% MTBE:hexanes; purified using automated flash column chromatography using an MTBE:hexanes gradient mobile phase employing a 2.5% isocratic hold; isolated yield 0.049 g, 60%; beige solid; mp = 186.0–187.5 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.20 (s, 1H), 7.22–7.18 (m, 2H), 6.72 (br-d, J = 8.5 Hz, 2H), 2.98 (s, 6H), 2.86–2.81

(m, 2H), 2.77–2.72 (m, 2H), 1.98 (pent, $J = 7.5$ Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 150.2, 148.8, 148.6, 129.22, 129.2X (overlaps with 129.22), 112.22, 40.6, 38.2, 33.1, 22.0; IR (ATR-$CDCl_3$): $\overline{\upsilon}_{max} = 3195$, 2953, 2853, 1610, 1521, 1359, 965, 905, 727 cm^{-1}; HRMS (EI): *m/z* calculated for $C_{14}H_{18}N_2O$: 230.1419; found: 230.1425.

4-[2-(Hydroxyimino-methyl)-cyclopent-1-enyl]-*N*-(2-methoxy-ethyl)-benzamide (8q): Prepared according to the general procedure discussed above with 4-(2-formyl-cyclopent-1-enyl)-*N*-(2-methoxy-ethyl)-benzamide (0.37 mmol) and hydroxylamine hydrochloride, $R_F = 0.32$, 75% EtOAc:hexanes; purified using automated flash column chromatography using an EtOAc:hexanes gradient mobile phase; isolated yield 0.050 g, 48%; white film; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.33 (br-s, 1H), 8.07 (s, 1H), 7.80–7.70 (m, 2H), 7.35–7.30 (m, 2H), 6.65 (br-t, $J = 5.0$ Hz, 1H), 3.70–3.65 (m, 2H), 3.60–3.56 (m, 2H), 3.41 (s, 3H), 2.89–2.84 (m, 2H), 2.79–2.74 (m, 2H), 2.02 (pent, $J = 7.5$ Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 167.2, 147.5, 147.1, 140.0, 133.7, 133.0, 128.3, 127.2, 71.4, 59.0, 38.8, 38.4, 32.3, 22.1; IR (ATR-$CDCl_3$): $\overline{\upsilon}_{max} =$ 3289, 3047, 2926, 2852, 1638, 1609, 1542, 1304, 1117, 966, 853, 732 cm^{-1}; HRMS (EI): *m/z* calculated for $C_{16}H_{20}N_2O_3$: 288.1474; found: 288.1479.

2-(4-Methylsulfanyl-phenyl)-cyclopent-1-enecarbaldehyde oxime (8r): Prepared according to the general procedure discussed above with **6p** (0.73 mmol, 1.00 equiv) and hydroxylamine hydrochloride, $R_F = 0.34$, 20% MTBE:hexanes; purified using automated flash column chromatography using an MTBE:hexanes gradient mobile phase employing a 10% isocratic hold; isolated yield 0.097 g, 57%; yellow solid; mp = 141.0–142.5 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.10 (s, 1H), 7.26–7.22 (m, 2H), 7.21–7.17 (m, 2H), 2.87–2.81 (m, 2H), 2.78–2.73 (m, 2H), 2.49 (s, 3H), 2.01 (pent, $J = 7.5$ Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 148.3, 148.2, 138.6, 133.4, 131.2, 128.6, 126.4, 38.3, 33.1, 22.1, 15.8; IR (ATR-$CDCl_3$): $\overline{\upsilon}_{max} =$ 3256, 3002, 2951, 2924, 2850, 1623, 1605, 1591, 965, 818 cm^{-1}; HRMS (EI): *m/z* calculated for $C_{13}H_{15}NOS$: 233.0874; found: 233.0875.

2-Benzo[1,3]dioxol-5-yl-cyclopent-1-enecarbaldehyde oxime (8s): Prepared according to the general procedure discussed above with **6q** (0.41 mmol, 1.00 equiv) and hydroxylamine hydrochloride, $R_F = 0.34$, 20% MTBE:hexanes; purified using automated flash column chromatography using an MTBE:hexanes gradient mobile phase employing a 5% isocratic hold; isolated yield 0.062 g, 65%; tan solid; mp = 139.0–141.5 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.10 (s, 1H), 6.82–6.79 (m, 1H), 6.77–6.74 (m, 2H), 5.98 (s, 2H), 2.84–2.79 (m, 2H), 2.77–2.71 (m, 2H), 2.00 (pent, $J = 7.5$ Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 148.5, 148.1, 147.8, 147.5, 130.7, 122.03, 122.0X (overlaps with 122.03), 108.5, 108.4, 101.3, 38.6, 33.1, 22.0; IR (ATR-$CDCl_3$): $\overline{\upsilon}_{max} =$ 3271, 2957, 2895, 2850, 1621, 1605, 1504, 1487, 1440, 1248, 1039, 936 cm^{-1}; HRMS (EI): *m/z* calculated for $C_{13}H_{13}NO_3$: 231.0895; found: 231.0889.

2-(3,3-Dimethyl-butyl)-cyclopent-1-enecarbaldehyde oxime (8t): Prepared according to the general procedure discussed above with 2-(3,3-dimethyl-butyl)-cyclopent-1-enecarbaldehyde (0.33 mmol) and hydroxylamine hydrochloride, $R_F = 0.55$, 20% MTBE:hexanes; purified using automated flash column chromatography using an MTBE:hexanes gradient mobile phase employing a 7.5% isocratic hold; isolated yield 0.032 g, 50%; amorphous; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.10 (s, 1H), 2.54 (br-t, $J = 7.5$ Hz, 2H), 2.45 (br-t, $J = 7.5$ Hz, 2H), 2.24–2.18 (m, 2H), 1.86 (pentet, $J = 7.5$ Hz, 2H), 1.31–1.24 (m, 2H), 0.92 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 152.2, 146.5, 128.6, 42.7, 37.2, 30.6, 29.3, 24.1, 21.9; IR (ATR-$CDCl_3$): $\overline{\upsilon}_{max} =$ 3290, 2953, 2866, 1639, 1601, 904, 727, 650 cm^{-1}; HRMS (EI): *m/z* calculated for $C_{12}H_{21}NO$: 195.1623; found: 195.1620.

2-Vinyl-cyclopent-1-enecarbaldehyde oxime (8u): Prepared according to the general procedure discussed above with 2-vinyl-cyclopent-1-enecarbaldehyde (0.50 mmol) and hydroxylamine hydrochloride, $R_F = 0.54$, 20% MTBE:hexanes; purified using automated flash column chromatography using an MTBE:hexanes gradient mobile phase employing a 7.5% isocratic hold; isolated yield 0.014 g, 20%; brown oil; ^{1}H NMR (500 MHz, $CDCl_3$) *major oxime diastereomer*: δ 8.28 (s, 1H), 6.81 (dd, $J = 17.0$, 10.0 Hz, 1H), 5.29 (d, $J = 17.5$ Hz, 1H), 5.27 (d, $J = 10.0$ Hz, 1H), 2.67 (t, $J = 7.5$ Hz, 2H), 2.62 (t, $J = 7.5$ Hz, 2H), 1.92 (pent, $J = 7.5$ Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) *major oxime diastereomer*: δ 146.4, 145.6, 133.2, 129.5, 117.5, 33.2, 32.8, 21.6; IR (ATR-$CDCl_3$): $\overline{\upsilon}_{max} =$ 3271, 3093, 2925, 2849, 1603, 1009, 992, 937, 909, 733 cm^{-1}; HRMS (EI): *m/z* calculated for $C_8H_{11}NO$ (M − H): 136.0757; found: 136.0765.

3-Phenyl-6,7-dihydro-5H-[2]pyrindine (8v): Material afforded as a byproduct to chromatographic purification of **8f** above. $R_F = 0.80$, 20% MTBE:hexanes; purified using automated flash column chromatography using an MTBE:hexanes gradient mobile phase employing a 5% isocratic hold; film; ^{1}H NMR (500 MHz, CD_3OD): δ 8.42 (s, 1H), 7.88–7.84 (m, 2H), 7.71 (s, 1H), 7.49–7.44 (m, 2H), 7.42–7.40 (m, 1H), 3.05–2.98 (m, 4H), 2.17 (pent, $J = 7.5$ Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 157.5, 157.0, 145.5, 140.8, 140.7, 129.8, 129.7, 128.2, 118.9, 33.7, 30.8, 26.2; IR (ATR-$CDCl_3$): $\overline{\upsilon}_{max} =$ 3201, 3029, 2847, 1606, 1556, 1475, 1448, 1073, 736, 694 cm^{-1}; HRMS (EI): *m/z* calculated for $C_{14}H_{13}N$: 195.1048; found: 195.1048.

2-Styryl-cyclohex-1-enecarbaldehyde oxime (9a): Prepared according to the general procedure discussed above with **7a** (0.54 mmol, 1.00 equiv) and hydroxylamine hydrochloride, $R_F = 0.50$, 20% MTBE:hexanes; purified using automated flash column chromatography using an MTBE:hexanes gradient mobile phase employing a 2.5% isocratic hold; isolated yield 0.061 g, 50%; white solid; mp = 142.0–143.0 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.61 (s, 1H), 7.47–7.43 (m, 2H), 7.39–7.32 (m, 3H), 7.28–7.23 (m, 1H), 6.70 (d, $J = 15.9$ Hz, 1H), 2.49–2.41 (m, 4H), 1.78–1.65 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 149.1, 139.1, 167.5, 129.6, 129.3, 128.8, 127.9, 126.7, 125.0, 26.9, 25.5, 22.4, 22.1; IR (ATR-$CDCl_3$): $\overline{\upsilon}_{max} =$ 3289, 3056, 2931, 2861, 1599, 1582, 1495, 950, 748, 691 cm^{-1}; HRMS (EI): *m/z* calculated for $C_{15}H_{17}NO$: 227.1310; found: 227.1304.

2-(2-*p*-Tolyl-vinyl)-cyclohex-1-enecarbaldehyde oxime (9b): Prepared according to the general procedure discussed above with **7b** (0.35 mmol, 1.00 equiv) and hydroxylamine hydrochloride, $R_F = 0.49$, 20% MTBE:hexanes; purified using automated flash column chromatography using an MTBE:hexanes gradient mobile phase employing a 7.5% isocratic hold; isolated yield 0.059 g, 69%; white solid; mp = 145.5–155.5 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.60 (s, 1H), 7.34 (d, $J = 8.0$ Hz, 2H), 7.32 (d, $J = 16.0$ Hz, 1H), 7.15 (d, $J = 8.0$ Hz, 2H), 6.67 (d, $J = 16.0$ Hz, 1H), 2.49–2.40 (br-m, 4H), 2.35 (s, 3H), 1.77–1.65 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 149.3, 139.3, 138.0, 134.7, 129.6, 129.6, 128.8, 126.7, 124.0, 26.9, 25.5, 22.4, 22.1, 21.4; IR (ATR-$CDCl_3$): $\overline{\upsilon}_{max} =$ 3249, 3053, 3017, 2931, 2861, 1611, 1578, 1444, 950, 800 cm^{-1}; HRMS (EI): *m/z* calculated for $C_{16}H_{19}NO$: 241.1467; found: 241.1465.

2-(2-*p*-Tolyl-vinyl)-benzaldehyde oxime (13a): Prepared according to the general procedure discussed above with **11a** (0.52 mmol, 1.00 equiv) and hydroxylamine hydrochloride, $R_F = 0.36$, 20% MTBE:hexanes; purified using automated flash column chromatography using an MTBE:hexanes gradient mobile phase employing a 10% isocratic hold; isolated yield 0.050 g, 41%; pale-yellow solid; mp = 125.7–127.3 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.55 (s, 1H), 7.70 (dd, $J = 7.7$, 1.3 Hz, 1*H*0, 7.67 (d, $J = 8.0$ Hz, 1H), 7.57 (br-s, 1H), 7.49–7.37 (m, 4H), 7.34–7.26 (m, 1H), 7.18 (d, $J = 7.7$ Hz, 2H), 6.96 (d, $J = 16.0$ Hz, 1H), 2.38 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 149.5, 138.2, 137.3, 134.5, 132.5, 130.1, 129.6, 129.5, 127.6, 126.9, 126.8, 124.6, 21.4; IR (ATR-$CDCl_3$): $\overline{\upsilon}_{max} =$ 3307, 3056, 3027, 2920, 1634, 1597, 1515, 1485, 1451, 1302, 961, 804, 753 cm^{-1}; HRMS (EI): *m/z* calculated for $C_{16}H_{15}NO$: (M − H) 236.1070; found: 236.1068.

2-[2-(4-Methoxy-phenyl)-vinyl]-benzaldehyde oxime (13b): Prepared according to the general procedure discussed above with **11b** (0.81 mmol, 1.00 equiv) and hydroxylamine hydrochloride, $R_F = 0.36$, 20% MTBE:hexanes; purified using automated flash column chromatography using an MTBE:hexanes gradient mobile phase; isolated yield 0.090 g, 65%; pale-yellow solid; mp = 142.3–144.2 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.52 (s, 1H), 7.67 (dd, ($J = 7.8$, 1.3 Hz, 1H), 7.59 (d, $J =$

7.8 Hz, 1H), 7.47–7.43 (m, 2H), 7.39–7.35 (m, 1H), 7.34 (d, $J = 16.3$ Hz, 1H), 7.28–7.26 (m, 1H), 6.95–6.88 (m, 3H), 3.83 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 159.7, 149.6, 137.4, 132.0, 130.0, 129.97, 129.3, 128.1, 127.5, 127.4, 126.7, 123.4, 114.2, 55.4; IR (ATR-$CDCl_3$): $\overline{\upsilon}_{max} = 3193$, 2990, 2966, 2912, 2838, 1603, 1511, 1246, 1176, 1030, 980, 959, 824, 811, 761, 546, 517 cm^{-1}; HRMS (EI): *m/z* calculated for $C_{16}H_{15}NO_2$: 253.1103; found: 253.1091.

4′-Methoxy-biphenyl-2-carbaldehyde oxime (13c): Prepared according to the general procedure discussed above with **11c** (0.65 mmol, 1.00 equiv) and hydroxylamine hydrochloride, $R_F = 0.39$, 20% MTBE: hexanes; purified using automated flash column chromatography using an MTBE:hexanes gradient mobile phase employing a 20% isocratic hold; isolated yield 0.074 g, 51%; amorphous; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.12 (s, 1H), 7.89 (7.3, 1.8 Hz, 1H), 7.42 (dt, $J = 7.5$, 1.3 Hz, 1H), 7.38–7.31 (m, 2H), 7.26–7.21 (m, 3H), 7.00–6.96 (m, 2H), 3.87 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 159.3, 150.2, 142.1, 132.0, 131.0, 130.5, 129.8, 129.75, 128.3, 127.4, 114.0, 55.1; IR (ATR-$CDCl_3$): $\overline{\upsilon}_{max} =$ 3299, 3068, 2835, 1610, 1515, 1482, 1442, 1298, 1245, 1178, 955, 834, 763 cm^{-1}; HRMS (EI): *m/z* calculated for $C_{14}H_{13}NO_2$: 227.0946; found: 227.0947.

2-Styryl-pyridine-3-carbaldehyde oxime (14a): Prepared according to the general procedure discussed above with **12a** (0.32 mmol, 1.00 equiv) and hydroxylamine hydrochloride, $R_F = 0.23$, 20% MTBE:hexanes; purified using automated flash column chromatography using an MTBE:hexanes gradient mobile phase employing a 30% isocratic hold; isolated yield 0.030 g, 42%; white solid; mp = 168.1–171.1 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.62 (dd, $J = 4.7$, 1.8 Hz, 1H), 8.54 (s, 1H), 7.98 (dd, $J = 8.0$, 1.8 Hz, 1H), 7.81 (d, $J = 15.6$ Hz, 1H), 7.61–7.57 (m, 2H), 7.56 (br-s, 1H), 7.49 (d, $J = 15.6$ Hz, 1H), 7.40–7.35 (m, 2H), 7.33–7.28 (m, 1H), 7.18 (dd, $J = 8.0$, 4.7 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 153.2, 150.6, 147.7, 136.8, 135.9, 135.4, 128.9, 128.8, 127.6, 125.3, 123.3, 122.3; IR (ATR-$CDCl_3$): $\overline{\upsilon}_{max} = 3058$, 2879, 2772, 1634, 1578, 1494, 904, 727, 650 cm^{-1}; HRMS (EI): *m/z* calculated for $C_{14}H_{12}N_2O$: (M − H) 223.0866; found: 223.0872.

2-(2-*p*-Tolyl-vinyl)-pyridine-3-carbaldehyde oxime (14b): Prepared according to the general procedure discussed above with **12b** (0.60 mmol, 1.00 equiv) and hydroxylamine hydrochloride, $R_F = 0.16$, 20% MTBE:hexanes; purified using automated flash column chromatography using an MTBE:hexanes gradient mobile phase employing a 35% isocratic hold; isolated yield 0.101 g, 72%; white solid; mp = 143.9–146.5 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.62 (dd, $J = 4.7$, 1.8 Hz, 1H), 8.56 (br-s, 1H), 7.99 (dd, $J = 7.9$, 1.8 Hz, 1H), 7.80 (d, $J = 15.9$ Hz, 1H), 7.53–7.48 (m, 3H), 7.45 (d, $J = 15.9$ Hz, 1H), 7.22–7.16 (m, 3H), 2.38 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 153.1, 149.9, 147.3, 139.2, 136.7, 135.9, 133.8, 129.7, 127.6, 125.5, 122.1, 121.5, 21.5; IR (ATR-$CDCl_3$): $\overline{\upsilon}_{max} = 2986$, 2883, 1636, 1581, 1510, 1477, 1407, 1066, 1058, 980, 812, 798 cm^{-1}; HRMS (EI): *m/z* calculated for $C_{15}H_{14}N_2O$: (M − H) 237.1022; found: 237.1029.

2-[2-(4-Methoxy-phenyl)-vinyl]-pyridine-3-carbaldehyde oxime (14c): Prepared according to the general procedure discussed above with **12c** (0.21 mmol, 1.00 equiv) and hydroxylamine hydrochloride, $R_F = 0.11$, 20% MTBE:hexanes; purified using automated flash column chromatography using an MTBE:hexanes gradient mobile phase employing a 15% isocratic hold; isolated yield 0.026 g, 47%; amorphous; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.59 (dd, $J = 4.7$, 1.7 Hz, 1H), 8.54 (s, 1H), 7.96 (dd, $J = 7.9$, 1.7 Hz, 1H), 7.77 (d, $J = 15.7$ Hz, 1H), 7.56–7.52 (m, 2H), 7.44 (br-s, 1H), 7.35 (d, $J = 15.7$ Hz, 1H), 7.15 (dd, $J = 7.9$, 4.7 Hz, 1H), 6.93–6.88 (m, 2H), 3.84 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 160.3, 153.6, 150.5, 147.6, 135.6, 135.3, 129.5, 129.0, 125.0, 121.9, 121.0, 114.3, 55.5; IR (ATR-$CDCl_3$): $\overline{\upsilon}_{max} = 3162$, 3064, 2837, 2771, 1632, 1605, 1575, 1511, 1427, 1253, 1174, 1031, 971, 826 cm^{-1}; HRMS (EI): *m/z* calculated for $C_{15}H_{14}N_2O_2$: 254.1055; found: 254.1052.

2-Phenyl-pyridine-3-carbaldehyde oxime (14d): Prepared according to the general procedure discussed above with **12d** (0.44 mmol, 1.00 equiv) and hydroxylamine hydrochloride, $R_F = 0.11$, 20% MTBE: hexanes; purified using automated flash column chromatography using an MTBE:hexanes gradient mobile phase employing a 25% isocratic hold; isolated yield 0.061 g, 70%; white solid; mp = 103.2–104.7 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.71 (dd, $J = 4.8$, 1.8 Hz, 1H), 8.22 (dd, $J = 8.0$, 1.8 Hz, 1H), 8.16 (br-s, 1H), 7.55–7.51 (m, 2H), 7.50–7.42 (m, 3H), 7.32 (dd, $J = 8.0$, 4.8 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 158.5, 150.5, 148.5, 138.6, 134.6, 129.8, 129.0, 128.6, 126.1, 122.5; IR (ATR-$CDCl_3$): $\overline{\upsilon}_{max} = 3060$, 2865, 1564, 1439, 1420, 976, 880, 747, 701 cm^{-1}; HRMS (EI): *m/z* calculated for $C_{12}H_{10}N_2O$: 198.0793; found: 198.0793.

2-*p*-Tolyl-pyridine-3-carbaldehyde oxime (14e): Prepared according to the general procedure discussed above with **12e** (0.22 mmol, 1.00 equiv) and hydroxylamine hydrochloride, $R_F = 0.21$, 20% MTBE: hexanes; purified using automated flash column chromatography using an MTBE:hexanes gradient mobile phase employing a 15% isocratic hold; isolated yield 0.030 g, 63%; white solid; mp = 203.6–206.2 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.70 (dd, $J = 4.7$, 1.7 Hz, 1H), 8.19 (dd, $J = 8.0$, 1.7 Hz, 1H), 8.17 (s, 1H), 7.45–7.41 (m, 2H), 7.35 (br-s, 1H), 7.30–7.27 (m, 3H), 2.43 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 158.6, 150.6, 148.9, 138.9, 135.8, 134.6, 129.8, 129.3, 125.8, 122.2, 21.5; IR (ATR-$CDCl_3$): $\overline{\upsilon}_{max} = 3163$, 3052, 2990, 2871, 2764, 1615, 1582, 1512, 1424, 977, 881, 827, 773 cm^{-1}; HRMS (EI): *m/z* calculated for $C_{13}H_{12}N_2O$: 212.0950; found: 212.0951.

2-(4-Methoxy-phenyl)-pyridine-3-carbaldehyde oxime (14f): Prepared according to the general procedure discussed above with **12f** (0.32 mmol, 1.00 equiv) and hydroxylamine hydrochloride, $R_F = 0.08$, 20% MTBE:hexanes; purified using automated flash column chromatography using an MTBE:hexanes gradient mobile phase employing a 10% isocratic hold; isolated yield 0.061 g, 84%; pale-yellow solid; mp = 208.4–212.1 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.71–8.68 (m, 1H), 8.21–8.16 (br-m, 2H), 7.52–7.47 (m, 2H), 7.30–7.26 (m, 1H), 7.05–6.98 (m, 2H), 3.88 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 160.4, 158.2, 150.6, 149.0, 134.7, 131.3, 131.1, 125.7, 122.0, 114.1, 55.5; IR (ATR-$CDCl_3$): $\overline{\upsilon}_{max} = 3163$, 3068, 2829, 2764, 1608, 1581, 1515, 1423, 1250, 1177, 903, 726 cm^{-1}; HRMS (EI): *m/z* calculated for $C_{13}H_{12}N_2O_2$: 228.0899; found: 228.0907.

3-Styryl-thiophene-2-carbaldehyde oxime (16a): Prepared according to the general procedure discussed above with **15a** (0.27 mmol, 1.00 equiv) and hydroxylamine hydrochloride, $R_F = 0.51$, 20% MTBE: hexanes; purified using automated flash column chromatography using an MTBE:hexanes gradient mobile phase employing a 25% isocratic hold; isolated yield 0.038 g, 62%; light-brown solid; mp = 132.9–135.1 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.56 (s, 1H), 7.51 (d, $J = 8.1$ Hz, 2H), 7.40–7.35 (m, 2H), 7.34–7.28 (m, 2H), 7.26–7.23 (m, 1H), 7.14 (s, 1H), 7.02 (d, $J = 16.2$ Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 144.1, 140.5, 137.0, 131.5, 130.4, 128.9, 128.3, 127.4, 126.7, 125.7, 120.0; HRMS (EI): *m/z* calculated for $C_{13}H_{11}NOS$: 229.0561; found: 229.0555.

3-(4-Methoxy-phenyl)-thiophene-2-carbaldehyde oxime (16b): Prepared according to the general procedure discussed above with **15b** (0.24 mmol, 1.00 equiv) and hydroxylamine hydrochloride, $R_F = 0.20$, 20% MTBE:hexanes; purified using automated flash column chromatography using an MTBE:hexanes gradient mobile phase employing a 15% isocratic hold; isolated yield 0.022 g, 38%; amorphous; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.70 (br-s, 1H), 7.55 (dd, $J = 5.8$, 0.7 Hz, 1H), 7.35–7.30 (m, 2H), 7.10 (d, $J = 5.0$ Hz, 1H), 6.99–6.95 (m, 2H), 3.85 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 159.5, 146.1, 141.3, 131.0, 130.4, 128.53, 128.45, 124.9, 114.2, 55.5; IR (ATR-$CDCl_3$): $\overline{\upsilon}_{max} = 3215$, 3100, 3002, 2932, 2841, 1608, 1575, 1528, 1249, 1178, 1031, 833 cm^{-1}; HRMS (EI): *m/z* calculated for $C_{12}H_{11}NO_2S$: 233.0510; found: 233.0511.

4.1. Minimum inhibitory concentration assays

4.1.1. Cell culture and in vitro functional assays

HEK293T cells were cultured in DMEM containing 10% FBS and 1% penicillin and streptomycin (P/S). To test the effects of the novel compounds on FGF-23-mediated activation of FGFR1/α-KL complex, HEK293T cells were transiently transfected with either empty

expression vector or full-length human α-KL along with the ERK luciferase reporter system[12] and *Renilla* luciferase-null as internal control plasmid. Transfections were performed by electroporation using Cell Line Nucleofector Kit® according to the manufacturer's protocol (Amaxa, Inc., Gaithersburg, MD). Thirty-six hours after transfection, the transfected cells were treated with each of the newly synthesized compound (10^{-5} M) or selected compound with a range of $10^{-9} \sim 10^{-4}$ M in the presence or absence of 1 nM FGF23 for IC_{50}. After 5 h, the cells were lysed and luciferase activities measured using a Synergy® H4 Hybrid Multi-Mode Microplate Reader (Winooski, VT, USA) and Promega® Dual-Luciferase Reporter Assay System (Madison, WI, USA). The IC_{50} values of the test compounds were obtained graphically from concentration-effect curves using Prism 5.0 (GraphPad Software Inc).

4.1.2. Cytotoxicity assays

HEK293T cells were cultured in DMEM containing 10% FBS and 1% penicillin and streptomycin (P/S). To test cytotoxicity of the selected compounds, HEK293T cells were seeded into 96-well plate at a density of 5×10^4/well. The compound toxicity was evaluated by measuring lactate dehydrogenase (LDH) activity released in the media 5 h after the test compound or vehicle exposure using the CytoTox96 nonradioactive assay (Promega) and quantitated by measuring wavelength absorbance at 490 nm. The LDH released from the cells exposed to different concentrations ($10^{-6} \sim 10^{-3}$ M) of the compound were normalized to the amount of LDH released from vehicle-treated cells receiving 10 μL of 10 × Lysis Solution (100%, maximum LDH release) and were corrected for baseline LDH released from vehicle-treated cells. The EC_{50} values of the test compounds were obtained graphically from concentration-effect curves using Prism 5.0 (GraphPad Software Inc).

4.2. Statistical analysis

We evaluated differences between two groups by unpaired *t*-test. All values are expressed as means ± S.D. All computations were performed using a commercial biostatistics software (GraphPad Software Inc. La Jolla, CA).

Author contributions

The manuscript was composed from experimental, theoretical, and written contributions of all authors. All authors have given approval to the final version of the manuscript.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Synthetic procedures for the construction of select aldehydes requisite to analogue formation, in addition to ^1H and ^{13}C NMR data for all analogues not previously disseminated in the primary literature. Automated flash column chromatograms for select compounds not previously reported. This material is available free of charge. Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmc.2020.115877.

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