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Discovery of oxime-bearing naphthalene derivatives as a novel structural type of Nrf2 activators

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ABSTRACT

Recent studies have demonstrated that oxidative stress insult is one of major causes of tumor formation. Therefore, identify the effective anti-oxidative agents as a preventive approach to stop cancer progression has widely explored. Although, many potent anti-oxidative ingredients in the natural products have been identified but the amount from the nature source hindrances the clinical application. Compound which can activate Nrf2 signaling pathway result unregulated the cellular antioxidant-responses has been demonstrated as an effective chemopreventive approach for cancer treatment. In the present study, certain oxime-bearing naphthalene derivatives were synthesized and evaluated for their Nrf2 activation and anti-proliferative activities. Results indicated (*E*)-1-(naphthalen-2-yloxy)propan-2-one oxime (**11**) which increased 2.04-fold Nrf2/ARE-driven luciferase activity was more active than its 1-substituted isomer **10** (1.17-fold) and *t*-BHQ (1.77-fold), the known Nrf2 activator. The activities were further increased by the replacement of the peripheral methyl group with the phenyl ring in which (*Z*)-2-(naphthalen-2-yloxy)-1-phenylethanone oxime (**13a**) exhibited 3.49-fold potency of the positive control. It is worth to mention that compounds **11**, **13a**, and **13b** which showed significant Nrf2 activation are non-cytotoxic to the tested cells with $IC_{50} > 50$ μM. This observation strongly suggested that these compounds can be used for chemoprevention. Mechanism studies indicated that these compounds were capable of inducing the phosphorylation of Nrf2 protein at serine 40 which led to the activation of the Nrf2 transcriptional activity.

1. Introduction

Cancer mortality remains the leading cause of death in Taiwan for the past 30 years and also the second mortality in the world. Recent studies clearly demonstrated that DNA damage caused by environmental carcinogen[1] and ischemic or hypoxic condition induced by unexpectedly oxidative stress from uncontrolled reactive oxygen species (ROS)[2] are the major reasons for the tumor formation. Chemoprevention is an important approach which utilizes non-toxic natural or synthetic substances to interfere with neoplastic development and mortality.[3] A substantial amount of evidence has shown that many small synthetic molecules or herbal products can exert chemopreventive effects.

Chronic redox imbalance is known to cause facilitated aging and develop a wide spectrum of diseases such as cancer, neurodegenerative disorders, and cardiovascular diseases. Nuclear factor E2-related factor 2 (Nrf2), a ~600 amino acid residue transcriptional factor which is expressed ubiquitously in most organs especially in the systems that receive external environmental stresses like gastrointestinal tract and skin. Generally, the Nrf2 protein is restrained in cytoplasm by the Kelch-like ECH-associated protein 1 (Keap1) with a short half-life of ~20 min.[4] While cellular oxidative stress or environmental carcinogens induce the modification of the Keap1 protein phosphorylated status, Nrf2 protein is then

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released from the complex. Free Nrf2 protein can spontaneously translocate to nucleus, forms heterodimers with small Maf protein and recognizes antioxidant-responsive element (ARE) for further transcription. Subsequently, different genes such as NAD(P)H:quinone oxidoreductase 1 (NQO1), glutathione peroxidase (GPx) and heme oxygenase-1 (HO-1) are produced for cellular protection.[5,6]

Beside the Keap1 function, the phosphorylated status of Nrf2 also governs the function of Nrf2. Multiple protein kinases (p44/42 MAP-kinase, p38 MAPK, JNK, PKC and PI3-kinase/Akt) are reported responsible for N-terminal serine phosphorylation of Nrf2 under oxidative stress, electrophiles and carcinogens insults.[7,8] The Nrf2 activation could reduce the sensitivity of cells toward toxins, oxidative and carcinogens insults by elevating antioxidant and detoxifying enzymes which could prevent the carcinogenesis process at the initiating stage. Drugs that activate Nrf2 mitigate the ability of tumor formation and recently many evidences have shown that Nrf2-activating drugs display a disease-preventing role in several chronic disorders including diabetes and neurodegenerative disorders.[5]

The naphthalene ring can be recognized in various biologically active compounds with clinical applications. The most noteworthy example is propranolol, an anti-angina drug, which consists of aromatic naphthalene ring and the propanolamine side chain. Duloxetine is another example of naphthalene derivative which blocks the transport proteins for both norepinephrine and serotonin and clinically used for the treatment of depression. Other examples of naphthalene derivatives, nafimidone and its oxime ester derivative (**1**), were found to possess anticonvulsant activity which can be further developed for the treatment of epilepsy.[9–11] Therefore, preparation and extensive biological evaluations on naphthalene derivatives have continuously attracted our attention.[12–14] Recently, we have synthesized and evaluated anti-proliferative activities of certain naphthalene derivatives.[15–18] Among them, *N*-(naphthalen-2-yl)-2-(2-oxo-1,2,3,4-tetrahydroquinolin-6-yloxy)-acetamide (**2**)[18] was the most active against NPC-TW01 with an IC_{50} value of 0.6 μM. On the other hand, we have also found that the introduction of oxime functional group into its ketone precursors significantly enhanced anti-proliferative activities of the respective parent compounds. Among all the flavone derivatives synthesized, (*Z*)-6-[2-hydroxyimino-2-(4-methoxyphenyl)ethoxy]-2-phenyl-4*H*-1-benzopyran-4-one (**3**)[15] exhibited the most potent anti-proliferative activities against the growth of SKHep1, HeLa, and SAS cancer cells with IC_{50} value of 0.8, 1.0, and 0.8 μM, respectively. The present study describes the synthesis and anti-proliferative evaluations of oxime-bearing naphthalene derivatives (Fig. 1) whose structures resemble compounds **3** in which the flavone scaffold is replaced with naphthalene ring.

Wang et al.[19] reported that in addition to the induction of ARE-driven gene expression by certain alkylating agents, anticancer drugs such as mitoxantrone (a cytotoxic antibiotic), etoposide (a topoisomerase 2 inhibitor), and cisplatin (a platin compound that cross-links DNA strands) were all able to induce luciferase reporter gene activity. The metabolic processes by which these agents act as inducers of ARE genes, and how they impinge on Keap1, is not known. Based on results which indicated that Nrf2 can be activated by certain anticancer agents, the Nrf2/ARE-driven luciferase activities of newly synthesized naphthalene derivatives have also been evaluated.

2. Chemistry

The preparation of oxime-containing naphthalene derivatives is illustrated in Scheme 1. Alkylation of 1-naphthol (**4**) with chloroacetone under basic conditions gave 1-(naphthalen-1-yloxy)propan-2-one (**6**)[12] which was then treated with NH_2OH to afford exclusively (*E*)-1-(naphthalen-1-yloxy)propan-2-one oxime (**10**) in a good overall yield. The configuration of the oxime moiety was determined by through-space nuclear Overhauser effect spectroscopy (NOESY), which revealed coupling connectivity to CH_3 protons. Accordingly, reaction of 1-(naphthalen-2-yloxy)propan-2-one (**7**)[12] with NH_2OH afforded exclusively (*E*)-1-(naphthalen-2-yloxy)propan-2-one oxime (**11**). Alkylation of 1-naphthol (**1**) with 2-(bromoacetyl)naphthalene under basic conditions gave 2-(naphthalen-1-yloxy)-1-(naphthalen-2-yl)ethanone (**8h**) in a yield of 94%. Preparation of compounds **8a**–**8e**, **8g**, **9a**–**9e**, and **9g** was previously reported.[12] Compound **8h** was then treated with NH_2OH to afford exclusively (*Z*)-2-(naphthalen-1-yloxy)-1-(naphthalen-2-yl)ethanone oxime (**12h**) in a good yield. The same synthetic procedure was applied for the synthesis of naphthalen-1-yl oximes **12a**–**12i**, and **12j**, and naphthalen-2-yl oxime derivatives **13a**–**13j** from their respective ketone precursors. The configuration of the oxime moiety was determined by the ^{13}C NMR spectra. The carbon of CH_2 which is anti to the oxime moiety (*E*-form) shifted downfield, while that of the syn isomer (*Z*-form) shifted upfield (for examples, δ 71.65 for **8h** shifted upfield to 59.03 for (*Z*)-**12h**; δ 71.47 for **8f** shifted upfield to 59.01 for (*Z*)-**12f**).[20]

3. Results and discussion

All the synthesized oxime-bearing naphthalene derivatives were evaluated in vitro against a panel of three cancer cell lines (NPC-TW 01, H226, and Jurkat) using MTT assay. The concentration that inhibited the growth of 50% of cells (IC_{50}) was determined from the linear portion of the curve by calculating the concentration of tested agent that reduced absorbance in treated cells, compared to control cells, by 50%. Results from Table 1 indicated except (*Z*)-1-(2,5-dimethoxyphenyl)-2-(naphthalen-2-yloxy)ethanone oxime (**13j**) showed noticeable anti-proliferative activity against all the tested human cancer cell lines, NPC-TW 01, H226, and Jurkat, with IC_{50} values of 16.7, 8.6, and 10.1 μM, respectively. Nevertheless, all other compounds are either very weakly

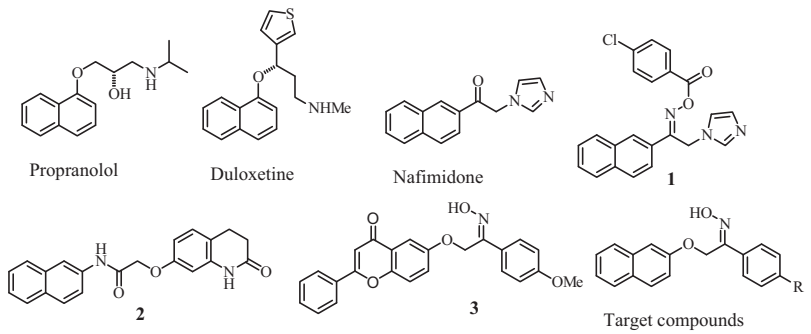
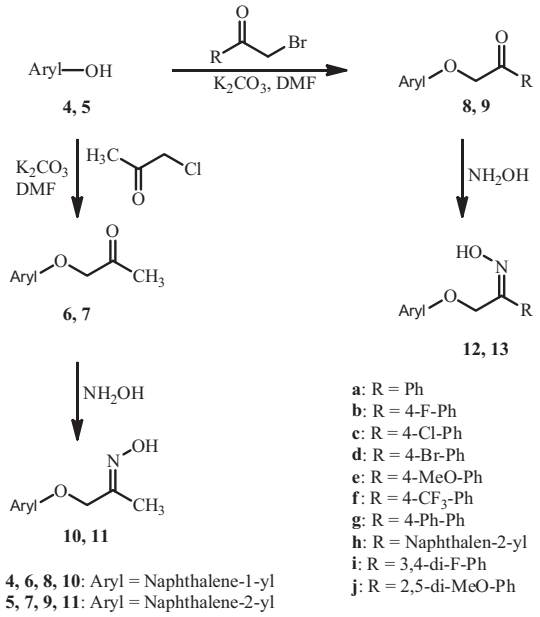
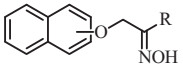


Figure 1. Structures of propranolol, duloxetine, nifimidone, compounds **1**–**3**, and target compounds.



Scheme 1.

Table 1
Antiproliferative activities of naphthalene derivatives

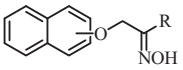


Compound	Position	R	IC_{50} (μM)		
			NPC-TW 01	H226	Jurkat
10	Naphthalene-1-yl	$-CH_3$	>50.0	>50.0	>50.0
11	Naphthalene-2-yl	$-CH_3$	>50.0	>50.0	>50.0
12a	Naphthalene-1-yl	Ph	>50.0	47.1	>50.0
12b	Naphthalene-1-yl	4-F-Ph	>50.0	>50.0	>50.0
12c	Naphthalene-1-yl	4-Cl-Ph	>50.0	>50.0	>50.0
12d	Naphthalene-1-yl	4-Br-Ph	>50.0	>50.0	>50.0
12e	Naphthalene-1-yl	4-OMe-Ph	32.3	36.7	31.8
12f	Naphthalene-1-yl	4-CF_3-Ph	46.4	>50.0	>50.0
12g	Naphthalene-1-yl	4-Ph-Ph	>50.0	>50.0	>50.0
12h	Naphthalene-1-yl	Naphthalene	36.1	38.9	>50.0
12i	Naphthalene-1-yl	3,4-F-Ph	36.8	>50.0	42.6
12j	Naphthalene-1-yl	2,5-OMe-Ph	32.3	41.5	>50.0
13a	Naphthalene-2-yl	Ph	>50.0	>50.0	>50.0
13b	Naphthalene-2-yl	4-F-Ph	>50.0	>50.0	>50.0
13c	Naphthalene-2-yl	4-Cl-Ph	26.0	34.9	37.3
13d	Naphthalene-2-yl	4-Br-Ph	27.9	39.1	47.2
13e	Naphthalene-2-yl	4-OMe-Ph	>50.0	>50.0	35.8
13f	Naphthalene-2-yl	4-CF_3-Ph	30.7	34.1	36.2
13g	Naphthalene-2-yl	4-Ph-Ph	>50.0	>50.0	>50.0
13h	Naphthalene-2-yl	Naphthalene	>50.0	37.1	24.3
13i	Naphthalene-2-yl	3,4-F-Ph	36.1	42.8	44.6
13j	Naphthalene-2-yl	2,5-OMe-Ph	16.7	8.6	10.1

(IC_{50} > 25 μM) or non-cytotoxic (IC_{50} > 50 μM) toward the tested cancer cells.

These oxime-bearing naphthalene derivatives were also subjected to the Nrf2-activation evaluation and results have shown in Table 2. Although majority of compounds exhibited only weak Nrf2 activation ability, (*E*)-1-(naphthalen-2-yloxy)propan-2-one oxime (**11**) which increased 2.04-fold Nrf2/ARE-driven luciferase activity was more active than its 1-substituted isomer **10** (1.17-fold) and the known Nrf2 activator, *tert*-butylhydroquinone (*t*-BHQ) (1.77-fold).[21–23] The activities were further increased by the replacement of the peripheral methyl group with the phenyl ring in which (*Z*)-2-(naphthalen-2-yloxy)-1-phenylethanone

Table 2
Naphthalene derivatives and tBHQ (20 μM) on Nrf2/ARE-driven luciferase activity



Compound	Position	R	Luciferase activity
10	Naphthalene-1-yl	$-CH_3$	1.17
11	Naphthalene-2-yl	$-CH_3$	2.04
12a	Naphthalene-1-yl	Ph	1.14
12b	Naphthalene-1-yl	4-F-Ph	1.09
12c	Naphthalene-1-yl	4-Cl-Ph	1.00
12d	Naphthalene-1-yl	4-Br-Ph	1.03
12e	Naphthalene-1-yl	4-OMe-Ph	1.10
12f	Naphthalene-1-yl	4-CF_3-Ph	1.01
12g	Naphthalene-1-yl	4-Ph-Ph	1.19
12h	Naphthalene-1-yl	Naphthalene	1.25
12i	Naphthalene-1-yl	3,4-F-Ph	0.86
12j	Naphthalene-1-yl	2,5-OMe-Ph	0.93
13a	Naphthalene-2-yl	Ph	3.49
13b	Naphthalene-2-yl	4-F-Ph	2.61
13c	Naphthalene-2-yl	4-Cl-Ph	1.07
13d	Naphthalene-2-yl	4-Br-Ph	0.95
13e	Naphthalene-2-yl	4-OMe-Ph	1.65
13f	Naphthalene-2-yl	4-CF_3-Ph	1.05
13g	Naphthalene-2-yl	4-Ph-Ph	1.18
13h	Naphthalene-2-yl	Naphthalene	1.36
13i	Naphthalene-2-yl	3,4-F-Ph	1.42
13j	Naphthalene-2-yl	2,5-OMe-Ph	0.65
t-BHQ	(Positive control)		1.77
Control			1.00

tert-Butylhydroquinone (*t*-BHQ).

oxime (**13a**) exhibited 3.49-fold potency of the control. However, further substitution on the phenyl ring decreased activities and the potency decreased in an order of **13b** (4-F, 2.61) > **13e** (4-MeO, 1.65) > **13i** (3,4-di-F, 1.42); **13h** (4-Ph, 1.36). The high steric hindrance of phenyl ring caused by either bulky group or di-functional group substitution is unfavorable for Nrf2 activation. It is worth to mention that compounds **11**, **13a**, and **13b** which showed more potent Nrf2 activation than the positive *t*-BHQ are non-cytotoxic to the tested cells with IC_{50} > 50 μM (Table 1). The observation clearly indicated that these compounds might be used for chemoprevention without any cytotoxic effect. However, compound **13j** (2,5-dimethoxy derivative) is the only compound that can slow down the cell proliferation (Table 1) and also paradoxically, inhibits Nrf2-driven luciferase activity.

To further explore the Nrf2-signaling activation mechanisms of oxime-bearing naphthalene, the expression level and phosphorylation status of Nrf2 protein were monitored. The TW01-pGL4-9ARE cells were treated with selected compounds (**11**, **13a**, **13b**, and **13e**) which activated Nrf2-signaling pathway in the cell-based luciferase assay and the phosphorylated level of Nrf2 at serine 40 which contributes to Nrf2 transcriptional activation[24] was observed. Results from Figure 2 indicated that although these compounds could not up-regulated the expression level of Nrf2 protein but are capable to induce the phosphorylation of Nrf2 protein at

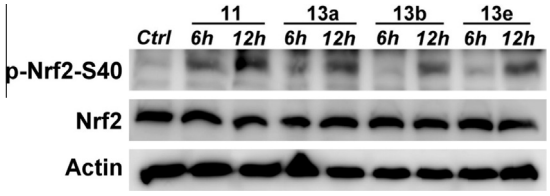


Figure 2. Naphthalene derivatives induced the phosphorylation of Nrf2 protein at serine 40. Treated TW01-pGL4-9ARE cells were harvested at indicated time and total cell lysates were extracted for Western blot analysis. After separation and transfer, the blots were incubated with antibodies specifically against p-Nrf2-S40, total Nrf2 or actin. Finally, the images were acquired by a biomolecular imager.

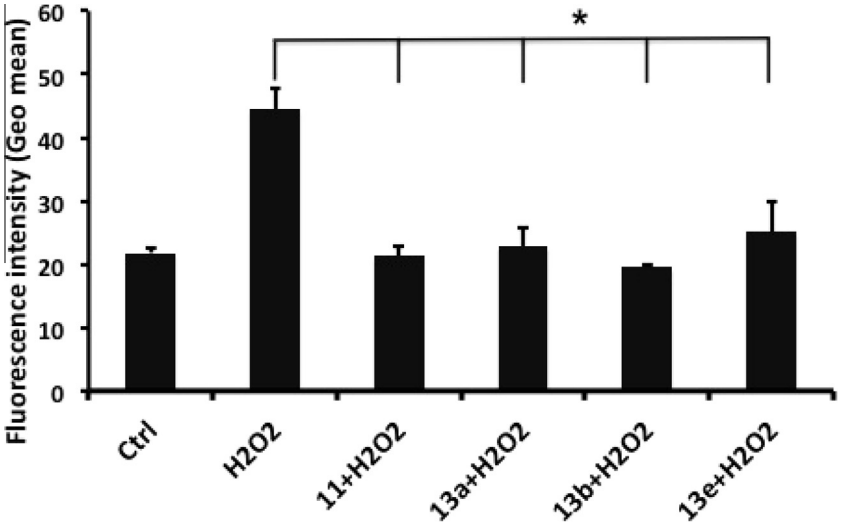


Figure 3. The pre-treatment of oxime-containing naphthalenes diminished H_2O_2-induced free radical production. TW01-pGL4-9ARE cells were seeded into 6-well plates (1×105 cells per well) and grown overnight. Subsequently, the cells were pre-treated with oxime-containing naphthalenes (20 μM) for 6 h incubation, followed by incubation of hydrogen peroxide (500 μM) for additional 16 h. Finally, DCFH-DA staining was utilized to detect intracellular free radical level and data were presented as geometric mean (Geo Mean) in each group. Asterisk (*) indicated significant difference between the co-treated group and H_2O_2-treated group (p <0.05).

Ser-40 position, which led to the activation of the Nrf2 transcriptional activity. Further experiments also clearly demonstrated that pre-treatment of compounds **11**, **13a**, **13b**, and **13e** could diminish the H_2O_2-induced intracellular oxidative stress, implying the ROS scavenging and chemopreventive capabilities of the oxime-bearing naphthalenes (Fig. 3). Therefore, the specific molecular target(s) of these oxime-bearing naphthalenes are worthy of further study. Our preliminary results demonstrated that staurosporine, a PKC signal-pathway inhibitor, significantly diminished the Nrf2-activation effects of oxime-containing naphthalenes, suggested that the PKC signaling pathways might involve in the oxime-containing naphthalenes-induced Nrf2 activation. However, specific molecular target(s) of these naphthalenes are currently under active investigation.

4. Conclusion

Many studies have showed that compounds that activate Nrf2-regulated cellular anti-oxidative responses could play a very important disease-preventing role in several chronic disorders including diabetes and neurodegenerative disorders and mitigate the ability of tumor formation.[5] In this study, certain oxime-bearing naphthalene derivatives were synthesized and evaluated for their Nrf2 activation and anti-proliferative activities. Among them, compound **13e** was less active while compounds **11**, **13a**, and **13b** showed more potent Nrf2 activation than the positive *t*-BHQ. Mechanism studies indicated that these compounds were capable of inducing the phosphorylation of Nrf2 protein at serine 40 which led to the activation of the Nrf2 transcriptional activity. To the best of our knowledge, this is the first study showed that compounds contain the naphthalene pharmacophore have the chemo-preventive activity through the activation the Nrf2-signaling pathway. Detail structure activity relationship and molecular mechanisms of activating the Nrf2-signaling pathway of oxime-bearing naphthalene are currently investigated.

5. Experimental

5.1. General

Melting points were determined on an Electrothermal IA9100 melting point apparatus and are uncorrected. Nuclear magnetic resonance (^{1}H and ^{13}C) spectra were recorded on a Varian-Unity-400 spectrometer. Chemical shifts were expressed in parts per million (δ) with tetramethylsilane (TMS) as an internal standard. Thin-layer chromatography was performed on silica gel 60 F-254 plates purchased from E. Merck and Co. The elemental analyses were performed in the Instrument Center of National Science Council at National Cheng-Kung University using Heraeus CHN-O Rapid EA, and all values are within ±0.4% of the theoretical compositions.

5.1.1. 2-(Naphthalen-1-yloxy)-1-(naphthalen-2-yl)ethanone (8h)

1-Naphthol (**4**, 1.44 g, 10 mmol), K_2CO_3 (1.38 g, 10 mmol), and dry DMF (50 mL) were stirred at room temperature (rt) for 30 min. To this solution was added 2-(bromoacetyl)naphthalene (2.49 g, 10 mmol) in DMF (10 mL) in one portion. The resulting mixture was stirred continuously at rt for 24 h (TLC monitoring) and then poured into ice-water (100 mL). The white solid thus obtained was collected and crystallized from Et_2O to give **8h** (2.92 g, 94%). Mp: 158–159 °C; ^{1}H NMR (400 MHz, $CDCl_3$-d_6): δ 5.56 (s, 2H, OCH_2), 6.86 (dd, J = 0.8, 7.6 Hz, 1H-C(2)), 7.37 (t, J = 7.6 Hz, 1H-C(3)), 7.48–7.66 (m, 4H, Ar-H), 7.81–7.84 (m, 1H, Ar-H), 7.90–7.99 (m, 3H, Ar-H), 8.13 (dd, J = 1.6, 8.8, 1H-C(5)), 8.41 (m, 1H, Ar-H), 8.65 (d, J = 1.2 Hz, 1H-C(8)); ^{13}C NMR (400 MHz, $CDCl_3$-d_6): δ 71.65 (CH_2O), 105.53 (C(2)), 121.57, 122.37, 124.01, 125.78, 125.80, 125.85, 126.84, 127.22, 127.68, 128.1, 128.97, 130.51, 132.25, 132.67, 134.84, 136.15, 154.06 (C(1)), 194.82 (C=O). Anal. calcd for $C_{22}H_{16}O_2$: C, 84.59; H, 5.16; found: C, 84.52; H, 5.26.

The same reaction procedures were applied for the preparation of **8f**, **8i**, and **8j** from compound **4**; compounds **9f**, **9i**, and **9j** from 2-naphthol (**5**).

5.1.2. 2-(Naphthalen-1-yloxy)-1-(4-(trifluoromethyl)phenyl)ethanone (8f)

81% yield. Mp: 91–92 °C; ^{1}H NMR (400 MHz, $CDCl_3$-d_6): δ 5.40 (s, 2H, OCH_2), 6.79 (d, J = 7.6 Hz, 1H-C(2)), 7.35 (t, J = 8 Hz, 1H-C(3)), 7.47–7.54 (m, 3H, Ar-H), 7.75–7.82 (m, 3H, Ar-H), 7.82 (dd, J = 2.8, 8 Hz, 1H-C(5)), 8.19 (dd, J = 0.8, 8.8 Hz, 2H, Ar-H), 8.30 (dd, J = 2.8, 9.2 Hz, 1H-C(8)); ^{13}C NMR (400 MHz, $CDCl_3$-d_6): δ 71.47 (CH_2O), 105.2 (C(2)), 121.63, 121.91, 125.48, 125.68, 125.79, 125.83, 125.87, 125.91, 126.73, 127.52, 128.83, 134.61, 137.37, 153.52 (C(1)), 194.21 (C=O). Anal. calcd for $C_{19}H_{13}F_3O_2$: C, 69.09; H, 3.97; found: C, 69.05; H, 3.99.

5.1.3. 1-(3,4-Difluorophenyl)-2-(naphthalen-1-yloxy)ethanone (8i)

86% yield. Mp: 139–140 °C; ^{1}H NMR (400 MHz, $CDCl_3$-d_6): δ 5.33 (s, 2H, OCH_2), 6.70 (d, J = 7.6 Hz, 1H-C(2)), 7.24–7.36 (m, 2H, Ar-H), 7.47–7.53 (m, 3H, Ar-H), 7.80–7.83 (m, 1H, Ar-H), 7.85–7.95 (m, 2H, Ar-H), 8.30 (dd, J = 2.8, 9.2 Hz, 1H-C(8)); ^{13}C NMR (400 MHz, $CDCl_3$-d_6): δ 71.33 (CH_2O), 105.19 (C(2)), 117.69, 117.81, 117.88, 118.0, 118.02, 121.62, 121.86, 125.48, 125.57, 125.61, 125.65, 125.68, 126.71, 127.52, 134.6, 153.47 (C(1)), 192.067 (C=O). Anal. calcd for $C_{18}H_{12}F_2O_2$: C, 72.48; H, 4.05; found: C, 72.35; H, 4.05.

5.1.4. 1-(2,5-Dimethoxyphenyl)-2-(naphthalen-1-yloxy)ethanone (8j)

73% yield. Mp: 128–129 °C; ^{1}H NMR (400 MHz, $CDCl_3$-d_6): δ 3.81 (s, 3H, OMe), 3.90 (s, 3H, OMe), 5.42 (s, 2H, OCH_2), 6.70 (d, J = 7.2 Hz, 1H-C(2)), 6.97 (d, J = 8.8, 1H, Ar-H), 7.13 (dd, J = 3.2, 8.8 Hz, 1H, Ar-H), 7.32 (t, J = 8 Hz, 1H-C(3)), 7.43–7.50 (m, 4H, Ar-H), 7.81 (dd, J = 1.6, 9.6 Hz, 1H-C(5)), 8.39 (dd, J = 2, 9.6 Hz, 1H-C(8)); ^{13}C NMR (400 MHz, $CDCl_3$-d_6): δ 55.84 (MeO), 56.8 (MeO), 74.62 (CH_2O), 105.24 (C(2)), 112.99, 113.8, 120.8, 121.71, 122.35, 125.24, 125.28, 125.56, 125.74, 126.44, 127.33, 134.59, 153.77, 153.89, 154.21 (C(1)), 195.03 (C=O). Anal. calcd for $C_{20}H_{18}O_4$: C, 74.52; H, 5.63; found: C, 74.24; H 5.66.

5.1.5. 2-(Naphthalen-1-yloxy)-1-(4-(trifluoromethyl)phenyl)ethanone (9f)

83% yield. Mp: 91–92 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.77 (s, 2H, OCH_2), 7.30 (d, J = 2.8, 8.8 Hz, 1H-C(3)), 7.38 (ddd, J = 1.2, 7.4, 1.2 Hz, 1H, Ar-H), 7.41 (d, J = 2.8 Hz, 1H-C(1)), 7.48 (ddd, J = 1.2, 7.4, 1.2 Hz, 1H, Ar-H), 7.78 (d, J = 8.4 Hz, 1H, Ar-H), 7.84–7.87 (m, 2H, Ar-H), 7.99 (d, J = 8.0 Hz, 2H, Ar-H), 8.28 (d, J = 8.0 Hz, 1H-C(5), 1H-C(8)); ^{13}C NMR (400 MHz, DMSO-d_6): δ 70.46 (CH_2O), 107.35 (C(1)), 118.51, 123.8, 125.78, 125.82, 125.85, 125.89, 126.5, 126.68, 127.56, 128.7, 128.85, 129.4, 132.85, 133.17, 134.12, 137.66, 155.69 (C(2)), 193.99 (C=O). Anal. calcd for $C_{19}H_{13}F_3O_2$: C, 69.09; H, 3.97; found: C, 69.07; H, 4.05.

5.1.6. 1-(3,4-Difluorophenyl)-2-(naphthalen-2-yloxy)ethanone (9i)

66% yield. Mp: 122–123 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.70 (s, 2H, OCH_2), 7.29 (dd, J = 2.8, 8.8 Hz, 1H-C(3)), 7.34–7.39 (m, 2H, Ar-H), 7.48 (ddd, J = 1.2, 7.6, 1.6, 1H, Ar-H), 7.66–7.77 (m, 2H, Ar-H), 7.84–7.87 (dd, J = 4.0, 8.0, 2H, Ar-H), 8.00 (m, 1H, Ar-H), 8.18 (m, 1H, Ar-H); ^{13}C NMR (400 MHz, DMSO-d_6): δ 70.23 (CH_2O), 107.35 (C(1)), 117.39, 117.56, 118.12, 118.29, 118.5, 123.8, 125.83, 125.86, 125.9, 126.5, 126.65, 127.56, 128.69, 129.4, 131.86, 131.9, 131.94, 134.11, 148.27, 148.4, 150.74, 150.86, 151.63, 151.75, 154.15, 154.28, 155.7 (C(2)), 192.37 (C=O). Anal. calcd for $C_{18}H_{12}F_2O_2$: C, 72.48; H, 4.05; found: C, 72.51; H, 4.21.

5.1.7. 1-(2,5-Dimethoxyphenyl)-2-(naphthalen-2-yloxy)ethanone (9j)

70% yield. Mp: 128–129 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.75 (s, 3H, OMe), 3.95 (s, 3H, OMe), 5.43 (s, 2H, OCH_2), 7.21–7.28 (m, 5H, Ar-H), 7.37 (ddd, J = 1.2, 7.6, 1.6, 1H, Ar-H), 7.46 (ddd, J = 1.2, 7.4, 1.2 Hz, 1H, Ar-H), 7.77 (d, J = 8 Hz, 1H-C(8)), 7.86 (dd, J = 3.2, 8.8 Hz, 2H, Ar-H); ^{13}C NMR (400 MHz, DMSO-d_6): δ 55.6 (MeO), 56.51 (MeO), 73.33 (CH_2O), 107.09 (C(1)), 113.32, 114.24, 118.55, 120.9, 123.67, 124.96, 126.4, 126.71, 127.5, 128.59, 129.34, 134.11, 153.14 (C(2)), 153.56, 155.93, 194.78 (C=O). Anal. calcd for $C_{20}H_{18}O_4$: C, 74.52; H, 5.63; found: C, 74.25; H, 5.63.

5.1.8. (*E*)-1-(Naphthalen-1-yloxy)propan-2-one oxime (10)

A solution of 1-(naphthalen-1-yloxy)propan-2-one (**6**)[12] (0.2 g, 1 mmol) in EtOH (20 mL) was added a solution of hydroxylamine hydrochloride (0.14 g, 2 mmol) in EtOH (2 mL). The mixture was heated at reflux for 4 h (TLC monitoring) and evaporated to give a residual solid. The white solid thus obtained was collected, purified by flash column chromatography (FC; silica gel; *n*-hexane/EtOAc 5:1) to give **10** (0.21 g, 89%). liquid; ^{1}H NMR (400 MHz, DMSO-d_6): (400 MHz, DMSO-d_6): 1.95 (s, 3H, CH_3), 4.76 (s, 2H, OCH_2), 7.04 (d, J = 7.6 Hz, 1H-C(2)), 7.49–7.56 (m, 4H, Ar-H), 7.90 (dd, J = 2.0, 7.2 Hz, 1H-C(5)), 8.20 (dd, J = 1.6, 7.6 Hz, 1H-C(8)), 11.03 (s, 1H-NOH); ^{13}C NMR δ 11.82 (CH_3), 69.74 (CH_2O), 107.68 (C(2)), 118.90, 124.08, 126.76, 126.96, 127.79, 128.88, 129.67, 134.34, 152.41 (C(1)), 156.24 (C=NOH). Anal. calcd for $C_{13}H_{13}NO_2$: C, 72.54; H, 6.09; N, 6.51; found: C, 72.55; H, 6.13; N, 6.40.

5.1.9. (*E*)-1-(Naphthalen-2-yloxy)propan-2-one oxime (11)

From 1-(naphthalen-2-yloxy)propan-2-one (**7**) as described for **10**: 80% yield. Mp: 127–128 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.89 (s, 3H, CH_3), 4.68 (s, 2H, OCH_2), 7.22 (dd, J = 2.4, 2.8 Hz, 1H-C(3)), 7.34–7.38 (m, 2H, Ar-H), 7.49 (t, J = 1.2, 2.8, 0.8 Hz, 1H-C(7)), 7.77–7.85 (m, 1H-C(4), 1H-C(5), 1H-C(8)), 11.01 (s, 1H-NOH); ^{13}C NMR (400 MHz, DMSO-d_6): δ 11.61 (CH_3), 69.59 (CH_2O), 107.47 (C(1)), 118.70, 123.81, 126.51, 126.73, 127.58, 128.67, 129.42, 134.16, 152.0 (C(2)), 156.06 (C=NOH). Anal. calcd for $C_{13}H_{13}NO_2$: C, 72.54; H, 6.09; N, 6.51; found: C, 72.69; H, 6.15; N, 6.37.

5.1.10. (*Z*)-2-(Naphthalen-1-yloxy)-1-phenylethanone oxime (12a)

From 2-(naphthalen-1-yloxy)-1-phenylethanone (**8a**) as described for the preparation of **10**: 71% yield. Mp: 121–122 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.48 (s, 2H, OCH_2), 7.10 (d, J = 7.6 Hz, 1H-C(2)), 7.36–7.46 (m, 7H, Ar-H), 7.72 (d, J = 2.4 Hz, 1H, Ar-H), 7.74 (d, J = 7.6 Hz, 1H-C(4)), 7.85 (d, J = 8 Hz, 1H-C(5)), 7.92 (d, J = 8 Hz, 1H-C(8)), 11.98 (s, 1H-NOH); ^{13}C NMR (400 MHz, DMSO-d_6): δ 59.17 (CH_2O), 105.11(C(2)), 120.40, 121.27, 124.80, 125.36, 126.14, 126.48, 127.43, 128.27, 128.90, 134.02, 134.22, 153.04, 153.25 (C=NOH). Anal. calcd for $C_{18}H_{15}NO_2$: C, 77.96; H, 5.45; N, 5.05; found: C, 77.96; H, 5.53; N, 5.07.

5.1.11. (*Z*)-1-(4-Fluorophenyl)-2-(naphthalen-1-yloxy)ethanone oxime (12b)

From 1-(4-fluorophenyl)-2-(naphthalen-1-yloxy)ethanone (**8b**) as described for **10**: 67% yield. Mp: 144–145 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.48 (s, 2H, OCH_2), 7.09 (d, J = 7.2 Hz, 1H-C(2)), 7.21–7.23 (m, 2H, Ar-H), 7.40–7.50 (m, 4H, Ar-H), 7.77 (d, J = 6.8 Hz, 1H-C(4)), 7.78 (d, J = 2.0 Hz, 1H, Ar-H), 7.85 (d, J = 8.0 Hz, 1H-C(5)), 7.92 (d, J = 8.2 Hz, 1H-C(8)), 12.0 (s, 1H-NOH); ^{13}C NMR (400 MHz, DMSO-d_6): δ 59.16 (CH_2O), 105.22 (C(2)), 115.11, 115.33, 120.47, 121.22, 124.77, 125.41, 126.13, 126.49, 127.45, 128.65, 128.74, 130.65, 134.01, 152.28, 153.13 (C=NOH), 161.23, 163.67. Anal. calcd for $C_{18}H_{14}FNO_2$: C, 73.21; H, 4.78; N, 4.74; found: C, 73.10; H, 4.78; N, 4.47.

5.1.12. (*Z*)-1-(4-Chlorophenyl)-2-(naphthalen-1-yloxy)ethanone oxime (12c)

From 1-(4-chlorophenyl)-2-(naphthalen-1-yloxy)ethanone (**8c**) as described for **10**: 69% yield. Mp: 176–177 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.48 (s, 2H, OCH_2), 7.08 (d, J = 7.2 Hz, 1H-C(2)), 7.40–7.51 (m, 6H, Ar-H), 7.76 (dd, J = 2.8, 8.4 Hz, 1H, Ar-H, 1H-C(4)), 7.85 (d, J = 8.0 Hz, 1H-C(5)), 7.92 (d, J = 8.4 Hz, 1H-C(8)), 12.09 (s, 1H-NOH); ^{13}C NMR (400 MHz, DMSO-d_6): δ 58.88 (CH_2O), 104.98 (C(2)), 120.38, 121.09, 124.64, 125.32, 126.0, 126.38, 127.34, 128.14, 128.23, 132.91, 133.45, 133.90, 152.08, 152.98 (C=NOH).

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Anal. calcd for $C_{18}H_{14}ClNO_2$: C, 69.35; H, 4.53; N, 4.49; found: C, 69.26; H, 4.61; N, 4.42.

5.1.13. (*Z*)-1-(4-Bromophenyl)-2-(naphthalen-1-yloxy)ethanone oxime (12d)

From 1-(4-bromophenyl)-2-(naphthalen-1-yloxy)ethanone (**8d**) as described for **10**: 66% yield. Mp: 178–179 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.47 (s, 2H, OCH_2), 7.08 (d, J = 7.6 Hz, 1H-C(2)), 7.40–7.51 (m, 4H, Ar-H), 7.58 (d, J = 8.4 Hz, 2H, Ar-H), 7.68 (d, J = 7.6 Hz, 1H, Ar-H, 1H-C(4)), 7.85 (d, J = 8 Hz, 1H-C(5)), 7.92 (d, J = 8 Hz, 1H-C(8)), 12.10 (s, 1H-NOH); ^{13}C NMR (400 MHz, DMSO-d_6): δ 58.83 (CH_2O), 104.97 (C(2)), 120.38, 121.09, 122.18, 124.63, 125.33, 126.0, 126.34, 127.34, 128.40, 131.15, 133.27, 133.90, 152.18, 152.98 (C=NOH). Anal. calcd for $C_{18}H_{14}BrNO_2$: C, 60.69; H, 3.96; N, 3.93; found: C, 60.58; H, 4.00; N, 3.96.

5.1.14. (*Z*)-1-(4-Methoxyphenyl)-2-(naphthalen-1-yloxy)ethanone oxime (12e)

From 1-(4-methoxyphenyl)-2-(naphthalen-1-yloxy)ethanone (**8e**) as described for **10**: 82% yield. Mp: 129–130 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.74 (s, 3H, OCH_3), 5.45 (s, 2H, OCH_2), 6.94 (dd, J = 9.2, 2.4 Hz, 2H, Ar-H), 7.09 (d, J = 7.2 Hz, 1H-C(2)), 7.40–7.51 (m, 4H, Ar-H), 7.67 (d, J = 2.0 Hz, 1H, Ar-H), 7.69 (d, J = 6.8 Hz, 1H-C(4)), 7.85 (d, J = 8.0 Hz, 1H-C(5)), 7.96 (d, J = 8.0 Hz, 1H-C(8)), 11.77 (s, 1H-NOH); ^{13}C NMR (400 MHz, DMSO-d_6): δ 55.12 (MeO), 59.06 (CH_2O), 105.06 (C(2)), 113.70, 120.34, 121.31, 124.81, 125.38, 126.16, 126.47, 126.58, 127.44, 127.80, 134.02, 152.48, 153.27 (C=NOH), 159.80. Anal. calcd for $C_{19}H_{17}NO_3{\cdot}0.01H_2O$: C, 74.21; H, 5.57; N, 4.56; found: C, 73.68; H, 5.65; N, 4.53.

5.1.15. (*Z*)-2-(Naphthalen-1-yloxy)-1-(4-(trifluoromethyl)phenyl)-ethanone oxime (12f)

From 2-(naphthalen-1-yloxy)-1-(4-(trifluoromethyl)phenyl)-ethanone (**8f**) as described for **10**: 83% yield. Mp: 155–156 °C; ^{1}H NMR (400 MHz, DMSO-d_6): 5.53 (s, 2H, OCH_2), 7.10 (d, J = 7.6 Hz, 1H-C(2)), 7.38–7.50 (m, 4H, Ar-H), 7.76 (d, J = 8.4 Hz, 2H, Ar-H, 1H-C(4)), 7.85 (d, J = 8 Hz, 1H-C(5)), 7.90 (d, J = 8.2 Hz, Ar-H), 7.95 (d, J = 8 Hz, 1H-C(8)), 12.33 (s, 1H-NOH); ^{13}C NMR (400 MHz, DMSO-d_6): δ 59.01 (CH_2O), 105.15 (C(2)), 120.57, 121.16, 124.73, 125.13, 125.17, 125.21, 125.42, 125.49, 126.10, 126.49, 127.20, 127.45, 128.81, 129.13, 134.0, 138.18, 152.19, 153.07 (C=NOH). Anal. calcd for $C_{19}H_{14}F_3NO_2$: C, 66.09; H, 4.09; N, 4.06; found: C, 65.76; H, 4.10; N, 4.03.

5.1.16. (*Z*)-1-(Biphenyl-4-yl)-2-(naphthalen-1-yloxy)ethanone oxime (12g)

From 1-(biphenyl-4-yl)-2-(naphthalen-1-yloxy)ethanone (**8g**) as described for **10**: 70% yield. Mp: 182–183 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.51 (s, 2H, OCH_2), 7.13 (d, J = 7.2 Hz, 1H-C(2)), 7.35–7.50 (m, 7H, Ar-H), 7.70 (t, J = 7.2, 8.4 Hz, 1H-C(4)), 3H, Ar-H), 7.83 (d, J = 2.4 Hz, 2H, Ar-H), 7.85 (d, J = 8.4 Hz, 1H-C(5)), 7.98 (d, J = 8.0 Hz, 1H-C(8)), 12.04 (s, 1H-NOH); ^{13}C NMR (400 MHz, DMSO-d_6): δ 59.07 (CH_2O), 105.15 (C(2)), 120.44, 121.31, 124.82, 125.41, 126.17, 126.51, 126.59, 126.98, 127.45, 127.70, 128.99, 133.29, 134.03, 139.39, 140.47, 152.61, 153.29 (C=NOH). Anal. calcd for $C_{24}H_{19}NO_2$: C, 81.56; H, 5.42; N, 3.96; found: C, 81.39; H, 5.55; N, 3.96.

5.1.17. (*Z*)-2-(Naphthalen-1-yloxy)-1-(naphthalen-2-yl)ethanone oxime (12h)

From 2-(naphthalen-1-yloxy)-1-(naphthalen-2-yl)ethanone (**8h**) as described for **10**: 67% yield. Mp: 158–159 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.60 (s, 2H, OCH_2), 7.16 (d, J = 7.0 Hz, 1H-C(2)), 7.33–7.53 (m, 6H, Ar-H), 7.78–7.97 (m, 1H-C(4), 1H-C(5), 4H, Ar-H), 8.28 (s, 1H-C(8)), 12.11 (s, 1H-NOH); ^{13}C NMR (400 MHz, DMSO-d_6): δ 59.03 (CH_2O), 105.15 (C(2)), 120.42, 121.25, 123.76, 124.82, 125.36, 126.16, 126.23, 126.46, 126.67, 127.43, 127.51, 127.70, 128.34, 131.61, 132.65, 133.0, 134.03, 152.89, 153.24 (C=NOH). Anal. calcd for $C_{22}H_{17}NO_2$: C, 80.71; H, 5.23; N, 4.28; found: C, 80.58; H, 5.30; N, 4.27.

5.1.18. (*Z*)-1-(3,4-Difluorophenyl)-2-(naphthalen-1-yloxy)ethanone oxime (12i)

From 1-(3,4-difluorophenyl)-2-(naphthalen-1-yloxy)ethanone (**8i**) as described for **10**: 76% yield. Mp: 130–131 °C; ^{1}H NMR (400 MHz, DMSO-d_6): 5.48 (s, 2H, OCH_2), 7.08 (d, J = 7.6 Hz, 1H-C(2)), 7.40–7.58 (m, 6H, Ar-H), 7.77 (m, 1H, Ar-H, 1H-C(4)), 7.85 (d, J = 8 Hz, 1H-C(5)), 7.92 (d, J = 8 Hz, 1H-C(8)), 12.18 (s, 1H-NOH); ^{13}C NMR (400 MHz, DMSO-d_6): δ 58.98 (CH_2O), 105.18 (C(2)), 115.35, 115.53, 117.36, 117.54, 120.53, 121.1, 123.58, 123.61, 123.64, 123.68, 124.73, 125.43, 126.07, 126.48, 127.45, 134.0, 147.92, 148.04, 148.44, 148.58, 150.35, 150.47, 150.9, 151.0, 151.5, 152.98 (C=NOH). Anal. calcd for $C_{18}H_{13}F_2NO_2$: C, 69.01; H, 4.18; N, 4.47; found: C, 68.98; H, 4.20; N, 4.43.

5.1.19. (*Z*)-1-(2,5-Dimethoxyphenyl)-2-(naphthalen-1-yloxy)-ethanone oxime (12j)

From 1-(2,5-dimethoxyphenyl)-2-(naphthalen-1-yloxy)ethanone (**8j**) as described for **10**: 59% yield. Mp: 150–151 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.61 (s, 3H, MeO), 3.62 (s, 3H, MeO), 5.32 (s, 2H, OCH_2), 6.76 (d, J = 2.8 Hz, 1H, Ar-H)), 6.89 (d, J = 2.8 Hz, 1H, Ar-H), 6.91 (d, J = 7.6 Hz, 1H-C(2)), 6.93–6.96 (m, 2H, Ar-H), 7.29–7.46 (m, 4H, Ar-H), 7.61 (d, J = 8 Hz, 1H-C(5)), 7.81 (d, J = 8.4 Hz, 1H-C(8)), 11.68 (s, 1H-NOH); ^{13}C NMR (400 MHz, DMSO-d_6): δ 55.24 (MeO), 55.89 (MeO), 62.35 (CH_2O), 104.98 (C(2)), 112.13, 114.59, 115.47, 120.05, 121.04, 124.33, 124.70, 124.99, 126.01, 126.26, 127.22, 133.82, 151.60, 152.57, 153.44, 154.71 (C=NOH). Anal. calcd for $C_{20}H_{19}NO_4$: C, 71.20; H, 5.68; N, 4.15; found: C, 70.85; H, 5.71; N, 4.11.

5.1.20. (*Z*)-2-(Naphthalen-2-yloxy)-1-phenylethanone oxime (13a)

From 2-(naphthalen-2-yloxy)-1-phenylethanone (**9a**) as described for **10**: 63% yield. Mp: 143–144 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.48 (s, 2H, OCH_2), 7.10 (dd, J = 1.2, 7.6 Hz, 1H-C(3)), 7.34–7.50 (m, 7H, Ar-H), 7.71–7.74 (m, 1H, Ar-H, 1H-C(4)), 7.84 (d, J = 8 Hz, 1H-C(5)), 7.92 (d, J = 7.8 Hz, 1H-C(8)), 11.97 (s, 1H-NOH); ^{13}C NMR (400 MHz, DMSO-d_6): δ 59.15 (CH_2O), 105.09 (C(1)), 120.40, 121.27, 124.79, 125.36, 126.15, 126.48, 127.44, 128.27, 128.91, 134.02, 134.21, 153.03, 153.25 (C=NOH). Anal. calcd for $C_{18}H_{15}NO_2$: C, 77.96; H, 5.45; N, 5.05; found: C, 78.08; H, 5.45; N, 5.05.

5.1.21. (*Z*)-1-(4-fluorophenyl)-2-(naphthalen-2-yloxy)ethanone oxime (13b)

From 1-(4-fluorophenyl)-2-(naphthalen-2-yloxy)ethanone (**9b**) as described for **10**: 71% yield. Mp: 139–140 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.37 (s, 2H, OCH_2), 7.11 (dd, J = 2.4, 8.8 Hz, 1H-C(3)), 7.19–7.25 (m, 2H, Ar-H), 7.38 (t, J = 1.6, 2.4, 1.2, 1H, Ar-H), 7.42 (d, J = 2.4 Hz, 1H-C(4)), 7.49 (ddd, J = 1.2, 2.4, 1.2 Hz, 1H, Ar-H), 7.69–7.83 (m, 5H, Ar-H), 12.01 (s, 1H-NOH); ^{13}C NMR (400 MHz, DMSO-d_6): δ 58.86 (CH_2O), 106.97 (C(1)), 115.2, 115.42, 118.4, 123.85, 126.55, 126.74, 127.58, 128.58, 128.67, 128.71, 129.46, 130.76, 130.79, 134.14, 152.1, 155.73 (C=NOH), 161.29, 163.74. Anal. calcd for $C_{18}H_{14}FNO_2$: C, 73.21; H, 4.78; N, 4.74; found: C, 73.24; H, 4.83; N, 4.74.

5.1.22. (*Z*)-1-(4-Chlorophenyl)-2-(naphthalen-2-yloxy)ethanone oxime (13c)

From 1-(4-chlorophenyl)-2-(naphthalen-2-yloxy)ethanone (**9c**) as described for **10**: 71% yield. Mp: 142–143 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.37 (s, 2H, OCH_2), 7.10 (dd, J = 2.4, 8.8 Hz, 1H-C(3)),

7.38 (dd, J = 1.2, 1.6 Hz, 1H-(C1)), 7.41–7.49 (m, 4H, Ar-H), 7.71 (dd, J = 2.0, 8.0 Hz, 2H, Ar-H), 7.76–7.83 (m, 3H, Ar-H), 12.11 (s, 1H-NOH); ^{13}C NMR (400 MHz, DMSO-d_6): δ 58.69 (CH_2O), 106.93 (C(1)), 118.38, 123.83, 126.55, 126.72, 127.57, 128.19, 128.42, 128.69, 129.45, 133.12, 133.6, 134.1, 152.04, 155.67 (C=NOH). Anal. calcd for $C_{18}H_{14}ClNO_2$: C, 69.35; H, 4.53; N, 4.49; found: C, 69.38; H, 4.61; N, 4.51.

5.1.23. (*Z*)-1-(4-Bromophenyl)-2-(naphthalen-2-yloxy)ethanone oxime (13d)

From 1-(4-bromophenyl)-2-(naphthalen-2-yloxy)ethanone (**9d**) as described for **10**: 60% yield. Mp: 143–144 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.37 (s, 2H, OCH_2), 7.10 (dd, J = 2.4, 8.8 Hz, 1H-C(3)), 7.38 (dd, J = 0.8, 1.2 Hz, 1H-(C1)),7.42 (d, J = 2.8 Hz, 1H, Ar-H), 7.49 (dd, J = 0.8, 8.0 Hz, 1H, Ar-H), 7.57–7.64 (m, 4H, Ar-H), 7.76–7.83 (m, 3H, Ar-H), 12.12 (s, 1H-NOH); ^{13}C NMR (400 MHz, DMSO-d_6): δ 58.66 (CH_2O), 106.93 (C(1)), 118.38, 122.33, 123.85, 126.55, 126.73, 127.58, 128.47, 128.7, 129.46, 131.34, 133.49, 134.11, 152.14, 155.67 (C=NOH). Anal. calcd for $C_{18}H_{14}BrNO_2$: C, 60.69; H, 3.96; N, 3.93; found: C, 60.73; H, 3.96; N, 3.93.

5.1.24. (*Z*)-1-(4-Methoxyphenyl)-2-(naphthalen-2-yloxy)ethanone oxime (13e)

From 1-(4-methoxyphenyl)-2-(naphthalen-2-yloxy)ethanone (**9e**) as described for **10**: 73% yield. Mp: 129–130 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.76 (s, 3H, OCH_3), 5.34 (s, 2H, OCH_2), 6.94 (dd, J = 2.0, 8.8 Hz, 2H, Ar-H), 7.11 (dd, J = 2.4, 9.2 Hz, 1H-C(3)), 7.37 (dd, J = 1.2, 1.6 Hz, 1H-C(1)), 7.43–7.49 (m, 2H, Ar-H), 7.64 (dd, J = 2.0, 8.8 Hz, 1H-C(6), 1H-C(7)), 7.76–7.83 (m, 3H, Ar-H), (s, 1H-NOH); ^{13}C NMR (400 MHz, DMSO-d_6): δ 55.17 (MeO), 58.77 (CH_2O), 106.9 (C(1)), 113.80, 118.45, 123.79, 126.53, 126.73, 127.58, 128.67, 129.42, 134.17, 152.33, 155.87, 159.86 (C=NOH). Anal. calcd for $C_{19}H_{17}NO_3{\cdot}0.1H_2O$: C, 73.86; H, 5.55; N, 4.53; found: C, 73.61; H, 5.55; N, 4.47.

5.1.25. (*Z*)-2-(Naphthalen-2-yloxy)-1-(4-(trifluoromethyl)phenyl)ethanone oxime (13f)

From 2-(naphthalen-2-yloxy)-1-(4-(trifluoromethyl)phenyl)-ethanone (**9f**) as described for **10**: 66% yield. Mp: 131–132 °C; ^{1}H NMR (400 MHz, DMSO-d_6): 5.42 (s, 2H, OCH_2), 7.10 (dd, J = 2.4, 8.8 Hz, 1H-C(3)), 7.38 (dd, J = 1.2, 1.2 Hz, 1H-(C1)), 7.43–7.50 (m, 2H, Ar-H), 7.75–7.84 (m, 5H, Ar-H), 7.91 (d, J = 8.4 Hz, 2H, Ar-H), 12.34 (s, 1H-NOH); ^{13}C NMR (400 MHz, DMSO-d_6): δ 58.75 (CH_2O), 107.01 (C(1)), 118.38, 123.91, 125.25, 125.29, 125.32, 126.59, 126.76, 127.20, 127.60, 128.75, 129.51, 134.13, 138.29, 152.1, 155.66 (C=NOH). Anal. calcd for $C_{19}H_{14}F_3NO_2$: C, 66.09; H, 4.09; N, 4.06; found: C, 66.20; H, 4.43; N, 4.06.

5.1.26. (*Z*)-1-(Biphenyl-4-yl)-2-(naphthalen-2-yloxy)ethanone oxime (13g)

From 1-(biphenyl-4-yl)-2-(naphthalen-2-yloxy)ethanone (**9g**) as described for **10**: 64% yield. Mp: 183–184 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.41 (s, 2H, OCH_2), 7.14 (dd, J = 2.4, 8.8 Hz, 1H-C(3)), 7.34–7.40 (m, 2H, Ar-H), 7.45–7.50 (m, 4H, Ar-H), 7.68–7.71 (m, 4H, Ar-H), 7.78–7.84 (m, 5H, Ar-H), 12.05 (s, 1H-NOH); ^{13}C NMR (400 MHz, DMSO-d_6): δ 58.75 (CH_2O), 106.96 (C(1)), 118.46, 123.83, 126.55, 126.62, 126.74, 126.94, 127.58, 127.73, 128.70, 129.02, 129.46, 133.40, 134.18, 139.45, 140.52, 152.48, 155.87 (C=NOH). Anal. calcd for $C_{24}H_{19}NO_2$: C, 81.56; H, 5.42; N, 3.96; found: C, 81.73; H, 5.42; N, 3.97.

5.1.27. (*Z*)-1-(Naphthalen-2-yl)-2-(naphthalen-2-yloxy)ethanone oxime (13h)

From 1-(naphthalen-2-yloxy)-2-(naphthalen-2-yl)ethanone (**9h**) as described for **10**: 77% yield. Mp: 143–144 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.49 (s, 2H, OCH_2), 7.16 (dd, J = 2.4, 8.8 Hz, 1H-C(3)), 7.38 (dd, J = 1.2, 1.6 Hz, 1H-(C1)), 7.46–7.54 (m, 4H, Ar-H), 7.77–7.83 (m, 3H, Ar-H), 7.89–7.96 (m, 4H, Ar-H), 8.22 (s, 1H, Ar-H), 12.12 (s, 1H-NOH); ^{13}C NMR (400 MHz, DMSO-d_6): δ 58.7 (CH_2O), 107.0 (C(1)), 118.48, 123.74, 123.82, 126.11, 126.51, 126.55, 126.72, 126.75, 127.55, 127.58, 127.83, 128.41, 128.7, 129.44, 131.82, 132.72, 133.06, 134.18, 152.76, 155.9 (C=NOH). Anal. calcd for $C_{22}H_{17}NO_2$: C, 80.71; H, 5.23; N, 4.28; found: C, 80.86; H, 5.23; N, 4.28.

5.1.28. (*Z*)-1-(3,4-Difluorophenyl)-2-(naphthalen-2-yloxy)ethanone oxime (13i)

From 1-(3,4-difluorophenyl)-2-(naphthalen-2-yloxy)ethanone (**9i**) as described for **10**: 93% yield. Mp: 121–122 °C; ^{1}H NMR (400 MHz, DMSO-d_6): 5.37 (s, 2H, OCH_2), 7.11 (dd, J = 2.8, 8.8 Hz, 1H-C(3)), 7.38 (dd, J = 1.2, 1.6 Hz, 1H-(C1)), 7.42–7.54 (m, 4H, Ar-H), 7.67–7.84 (m, 4H, Ar-H), 12.19 (s, 1H-NOH); ^{13}C NMR (400 MHz, DMSO-d_6): δ 58.63 (CH_2O), 107.01 (C(1)), 115.23, 115.42, 117.45, 117.62, 118.33, 123.56, 123.83, 126.51, 126.7, 127.54, 128.7, 129.44, 134.07, 151.29, 155.58 (C=NOH). Anal. calcd for $C_{18}H_{13}F_2NO_2$: C, 69.01; H, 4.18; N, 4.47; found: C, 68.97; H, 4.21; N, 4.48.

5.1.29. (*Z*)-1-(2,5-Dimethoxyphenyl)-2-(naphthalen-2-yloxy)-ethanone oxime (13j)

From 1-(2,5-dimethoxyphenyl)-2-(naphthalen-2-yloxy)ethanone (**9j**) as described for **10**: 60% yield. liquid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.60 (s, 3H, MeO), 3.66 (s, 3H, MeO), 5.25 (s, 2H, OCH_2), 6.68 (d, J = 2.8 Hz, 1H, Ar-H)), 6.88–6.97 (m, 3H, Ar-H), 7.28 (d, J = 2.4 Hz, 1H, Ar-H), 7.36 (dd, J = 1.6, 8.0 Hz, 1H-(C3)), 7.47 (dd, J = 1.2, 1.6 Hz, 1H-(C1)), 7.73–7.81 (m, 3H, Ar-H), 11.73 (s, 1H-NOH); ^{13}C NMR (400 MHz, DMSO-d_6): δ 55.28 (MeO), 56.15 (MeO), 61.89 (CH_2O), 106.87 (C(1)), 112.44, 114.61, 115.61, 118.39, 123.65, 124.48, 126.39, 126.67, 127.47, 128.52, 129.24, 134.1, 151.62, 152.68, 154.61, 155.99 (C=NOH). Anal. calcd for $C_{20}H_{19}NO_4$: C, 71.20; H, 5.68; N, 4.15; found: C, 70.80; H, 5.69; N, 4.10.

5.2. Pharmacological methods

Materials: The antibody against p-Nrf2-S40 was purchased from Abcam (Cambridge, UK). The Nrf2 and actin antibodies were obtained from Santa Cruz Biotechnology (Dallas, Texas). Human T-cell leukemia (Jurkat) and non-small cell lung carcinoma (NCI-H226) cell lines were obtained from the American Type Culture Collection (Rockville, MD). A nasopharyngeal carcinoma (NPC-TW01) cell line was purchased from the Taiwan Food Industry Research and Development Institute, Hsinchu, Taiwan, Republic of China. All cells were grown in minimum essential medium (MEM) or RPMI1640 medium (Invitrogen) containing 10% fetal bovine serum (FBS) (Thermo), 100 units/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine and 1 mM sodium pyruvate (Invitrogen) and were cultured in a humidified incubator with 5% CO_2 set at 37 °C.

5.2.1. Cell viability

MTT assay was employed to determine the cell viability. Treated cells were incubated with MEM with 5 μg/ml MTT for 2 h at 37 °C. Afterward, the cells were further incubated with solubilization buffer (40% DMF and 20% SDS in H_2O) overnight at 37 °C. The absorbance at 570 nm was then detected by a microplate reader (Molecular Device).

5.2.2. Establishment of ARE-luciferase expressing cells

The antioxidant-responsive element reporter plasmids (pGL4-ARE9) were kindly provided by Dr. Ching-Chuan Kuo (National

Health Research Institutes, Tainan, Taiwan). NPC-TW01 or HONE-1 cells were electroporated with pGL4-ARE9 plasmids by electroporator (Thermo Electron Corp.). Subsequently, electroporated cells were maintained in completed MEM in the presence of 1 mg/ml G418 (Gibco) for at least four weeks. Single clones were then isolated and grown for further studies.

5.2.3. Luciferase activity assay

Cells (1×10^6 cells/well) were seeded into 12-well plates and incubated overnight, followed by drug treatment for additional 16 h. Treated cells were trypsinized and collected by centrifugation at $300 \times g$ for 10 min. Samples were then lyzed with lysis buffer (1% Triton X-100, 25 mM glycylglycine, 15 mM $MgSO_4$, 4 mM EGTA and 1 mM DTT) and centrifuged at $15000 \times g$ for 10 min. Finally, the supernatants were mixed with luciferase assay buffer (25 mM glycylglycine, 15 mM potassium phosphate, 15 mM $MgSO_4$, 4 mM EGTA, 2 mM ATP and 1 mM DTT) and luciferin solution (1 mM D-luciferin, 25 mM glycylglycine and 10 mM DTT). Luminescence was then measured by a luminometer.

5.2.4. Western blot analysis

Cells were treated and collected at indicated time. Cells were then lysed with cell lysis buffer (150 mM NaCl, 20 mM Tris–Cl pH 8.0, 0.5% NP-40, 1 mM PMSF, 1 mM NaF, 1 mM Na_3VO_4 and 20 μg/ml aprotinin in distilled H_2O) and protein concentration of each sample was measured by a BCA protein assay kit (Thermo). Total cell lysate (40 μg/well) was separated in 10% SDS–PAGE gels and then transferred to PVDF membranes. After blocking with 5% BSA/TBS-Tween, the membranes were reacted with primary antibodies overnight at 4 °C. Afterward, horseradish peroxidase-conjugated secondary antibodies were incubated with the membranes for 1 h at room temperature. After washing, the images were obtained on a LAS-4000 biomolecular imager (Fujifilm, Tokyo, Japan).

5.2.5. Detection of intracellular oxidative stress

To detect the level of intracellular oxidative stress, control and treated cells were incubated with 20 μM of DCFH-DA (2′,7′-dichlorodihydrofluorescein diacetate) for 30 min. at 37 °C prior to analysis. Cells were harvested and re-suspended in 1 × PBS and subjected to flowcytometry analysis on a Canto II cytometer. The acquired data was finally evaluated by the FCS express software.

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References and notes

1. Yager, J. D.; Davidson, N. E. *N. Eng. J. Med.* **2006**, *354*, 270.
2. Kim, J. W.; Tchernyshyov, I.; Semenza, G. L.; Dang, C. V. *Cell Metab.* **2006**, *3*, 177.
3. Rao, C. V.; Reddy, B. S. *Curr. Cancer Drug Targets* **2004**, *4*, 29.
4. Amakye, D.; Jagani, Z.; Dorsch, M. *Nat. Med.* **2013**, *19*, 1410.
5. Sporn, M. B.; Liby, K. T. *Nat. Rev. Cancer* **2012**, *12*, 564.
6. Niture, S. K.; Kaspar, J. W.; Shen, J.; Jaiswal, A. K. *Toxicol. Appl. Pharm.* **2010**, *244*, 37.
7. Adams, J.; Huang, P.; Patrick, D. *Biology* **2002**, *6*, 486.
8. Singh, S.; Vrishni, S.; Singh, B. K.; Rahman, I.; Kakkar, P. *Free Radical Res.* **2010**, *44*, 1267.
9. Walker, K. A.; Wallach, M. B.; Hirschfeld, D. R. *J. Med. Chem.* **1981**, *24*, 67.
10. Nardi, D.; Tajana, A.; Leonardi, A.; Pennini, R.; Portioli, F.; Magistretti, M. J.; Subissi, A. *J. Med. Chem.* **1981**, *24*, 727.
11. Karakurt, A.; Alagöz, M. A.; Sayoglu, B.; Çalıs, U.; Dalkara, S. *Eur. J. Med. Chem.* **2012**, *57*, 275.
12. Tzeng, C. C.; Lee, K. H.; Wang, T. C.; Han, C. H.; Chen, Y. L. *Pharm. Res.* **2000**, *17*, 715.
13. Chen, Y. L.; Wang, T. C.; Chang, N. C.; Chang, Y. L.; Teng, C. M.; Tzeng, C. C. *Chem. Pharm. Bull.* **1998**, *46*, 962.
14. Wang, T. C.; Lee, K. H.; Chen, Y. L.; Liou, S. S.; Tzeng, C. C. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2773.
15. Wang, T. C.; Chen, I. L.; Lu, P. J.; Wong, C. H.; Liao, C. H.; Tsiao, K. C.; Chang, K. M.; Chen, Y. L.; Tzeng, C. C. *Bioorg. Med. Chem.* **2005**, *13*, 6045.
16. Chen, L. C.; Juang, S. H.; Chang, K. M.; Tzeng, C. C.; Chen, J. J.; Chen, I. L.; Wang, T. C. *Chem. Pharm. Bull.* **2014**, *62*, 106.
17. Chen, I.-L.; Chen, J.-Y.; Shieh, P.-C.; Chen, J.-J.; Lee, C.-H. *Bioorg. Med. Chem.* **2008**, *16*, 7639.
18. Chen, I.-L.; Chen, J.-J.; Lin, Y.-C.; Peng, C.-T.; Juang, S.-H.; Wang, T.-C. *Eur. J. Med. Chem.* **2013**, *59*, 227.
19. Wang, X. J.; Hayes, J. D.; Wolf, C. R. *Cancer Res.* **2006**, *66*, 10983.
20. Silverstein, R. M.; Webster, F. X. ^{13}C NMR Spectrometry. In *Spectrometric Identification of Organic Compounds*; Rose, N., Ed., 6th ed.; John Wiley and Sons: New York, 1998; pp 217–249.
21. Shin, B. Y.; Jin, S. H.; Cho, I. J.; Ki, S. H. *Free Radical Biol. Med.* **2012**, *53*, 834.
22. Cheng, Q.; Kalabus, J. L.; Zhang, J.; Blanco, J. G. *Biochem. Pharm.* **2012**, *83*, 139.
23. Kang, K. W.; Cho, M. K.; Lee, C. H.; Kim, S. G. *Mol. Pharm.* **2001**, *59*, 1147.
24. Huang, H. C.; Nguyen, T.; Pickett, C. B. *J. Biol. Chem.* **2002**, *277*, 42769.