Tetrahedron 68 (2012) 10360–10364

Contents lists available at SciVerse ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Improved synthesis of 3-aryl isoxazoles containing fused aromatic rings

Yousef R. Mirzaei, Matthew J. Weaver, Scott A. Steiger, Alison K. Kearns, Mariusz P. Gajewski, Kevin C. Rider, Howard D. Beall, N.R. Natale *

Medicinal Chemistry Graduate Program, Department of Biomedical and Pharmaceutical Sciences, The University of Montana, Missoula, MT 59812, USA

ARTICLE INFO

Article history:
Received 14 August 2012
Received in revised form 14 September 2012
Accepted 17 September 2012
Available online 25 September 2012

ABSTRACT

A critical comparison of methods to prepare sterically hindered 3-aryl isoxazoles containing fused aromatic rings using the nitrile oxide cycloaddition (NOC) reveal that modification of the method of Bode, Hachisu, Matsuura, and Suzuki (BHMS), utilizing either triethylamine as base or sodium enolates of the diketone, ketoester, and ketoamide dipolarophiles, respectively, was the method of choice for this transformation.

1. Introduction

Isoxazoles continue to be of interest for both their biological activities and synthetic utility.[1–4] The synthesis of aryl-isoxazoles is also the subject of on-going improvements.[1,5–7] In connection with our studies on aryl isoxazole amides (AIMs)[8–9] we have reported lead compounds, which possess (1) useful antitumor activity and (2) photophysical properties, which are of potential diagnostic use as 'tumor paint'.[10]

An illustration of the application of AIMs is shown in Fig. 1. AIM NSC 728558 has exhibited a mid-graph mean point of −5.71–5.75 (log scale, which translate to single digit micromolar) for tumor cell line growth inhibition (GI_{50}) against the full panel in the NCI60 cell line antitumor screening protocol,[8] and as a benchmark for comparison clinically used agents 5-fluorodeoxyuridine, bleomycin and rubidazone gave values of −4.7, −5.2, and −5.8, respectively, in the same standard assay. The calculated physical property data for NSC 728558 fall within or close to values with useful bioavailability (radar graph, Symyx Draw v3.1; top right panel). Laser Scanning Cytometry shows that NSC 728558 does in fact permeate human glioma SNB-19 cells (the AIM is blue) and is localized within the nucleus. Surgical resection of many cancers, especially brain tumors or gliomas, have a poor success rate because of the difficulty of judging the border between cancerous and healthy tissue. Fluorescent agents with antitumor activity, such as the AIMs hold promise for improvements in visualizing tumors during surgery.[10b,c]

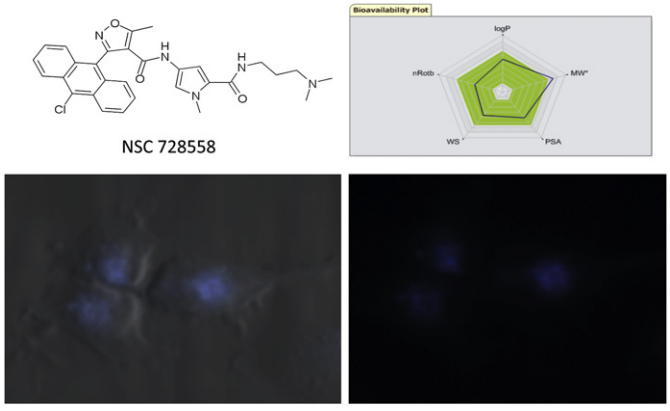
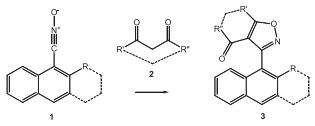


Fig. 1. Structure, Symyx radar graph, and Laser Scanning Cytometry of NSC 728558.

We required both improvements in the efficiency of the preparation of the isoxazole and concomitant economy of scale to prepare amounts of material sufficient to expand the scope of our investigations toward in vivo studies.



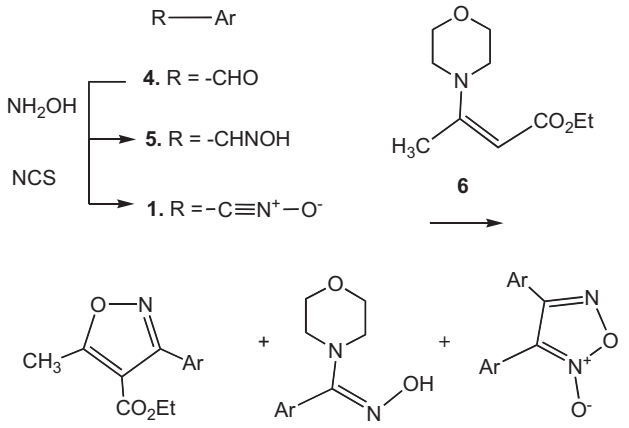
Isoxazoles prepared via nitrile oxide cycloaddition (NOC)[11] are most often prepared by dehydration of α-methylene nitro precursors,[5] or chlorination/dehalogenation of oximes[1,6,7] to form the nitrile oxide **1**.

Among the unsymmetrical dipolarophiles **2**, which usually give rise to regioselective cycloaddition are enamines. First pioneered by Stork and McMurry,[12] the use of enamines in the NOC works well for aliphatic and aromatic nitrile oxides on mole scales.[1] We have

* Corresponding author. Tel.: +1 406 243 4132; fax: +1 406 243 5228; e-mail address: nicholas.natale@umontana.edu (N.R. Natale).

0040-4020/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tet.2012.09.084

employed modifications of this synthesis in the preparation of isoxazole–oxazolines,[13] antihypertensive 4-isoxazolyl-1,4-dihydropyridines,[14] and we have championed the reaction as a learning tool for aspiring chemists.[15] Our previous reports in the AIM series have used enamines as dipolarophiles,[16] while for most cases modest yields in the range of 30–40% overall of **3** were obtained, sufficient quantities of material were isolated for pharmacology studies. Upon scale-up however, we have observed a consistent diseconomy of scale using enamines and nitrile oxides derived from fused ring aromatics, to the extent that even a ten-fold increase in scale (from ca. 10–100 mM) produced only marginally more desired product, and purified yields plummeted to 10–20%. *We have found this is especially a limitation when using nitrile oxides derived from fused aromatic systems*. Careful examination of the reaction by-products after chromatographic isolation and characterization by LC–MS revealed that as the stability of aryl nitrile oxides **1** increases[17,16b] their consequent reactivity to dipolarophiles slows, with concomitant increases in amine trapping of the nitrile oxide/oximidoyl chloride to give **7**, as well as self-condensation of the nitrile oxide to yield **8** as shown in Scheme 1.



Scheme 1. Synthesis of the isoxazoles used for the present study.

We therefore examined the methodology of Bode, Hachisu, Matsuura, and Suzuki (BHMS), which use either the sodium enolates of ketoesters,[6] or tertiary amines as base.[7] The use of sodium enolates in the NOC was pioneered by Renzi and Dal Piaz,[20] and recently applied in 2,6-disubstituted aryl cases for the preparation of growth hormone secretagogue receptor antagonists.[21] The BHMS procedure has been used to prepare particularly hindered unsymmetrical 2,6-disubstituted aryl examples, including examples, which appear to exist as atropisomers,[7] which recommended its application to our examples.

Using the BHMS procedure, the yields in all cases where critical comparison was made were dramatically improved (Table 1). Symmetrical diketones (Table 1, entries 1, 4, 5 and 11–13), unsymmetrical arylalkyl ketones (Table 1, entries 3, 9 and 10) and ketoesters (Table 1, entries 2, and 6–8) all produced the desired isoxazole products. In particularly hindered situations the triethyl amide protocol[7] often gave higher yields than the sodium ethoxide method.[6] (Table 1, entries 1, 3, 4 and 7, and compare entries 10 to 9), although in a select few cases we have observed that sodium isopropoxide to produce slightly higher yield (exemplified by Table 1, entries 11 and 14). All of the BHMS methods gave superior results to our experience using enamines as dipolarophiles for fused 3-aryl isoxazoles: for **3b** the BHMS conditions gave 70% (Table 1, entry 2) yield compared to 30–40% for the enamine method for which the by-products complicated purification and lowered the yield of desired isoxazole (Scheme 1). Similar results were observed for **3g** the yields were 80% (Table 1, entry 8) for the BHMS verses 35% for enamine (Scheme 1). In cases where the BHMS yields are modest, the enamine methodology gave only traces or no desired product at all that is, the enamine entries (not shown) corresponding to Table 1, entries 3 9,and 10, would correspond to traces at best).

The comparison was especially dramatic for the anthryl isoxazole cases, shown in Table 2. Excellent yields were obtained for anthryl isoxazoles **3j**, 10-chloro analog **3k**, and 10-bromo analog **3m**. The yields with the enamine procedures were 35–45[16] and 10–20%, for **3j** and **3k**, respectively. Table 2, entry 3 illustrates the direct synthesis of a fused aromatic containing a C-4 tertiary amide, which is an uncommon example of a ketamide used as dipolarophile in the NOC.[22] The ketamide was prepared from diketene and pyrrolidine,[18] and cycloaddition provided the C-4 amide in synthetically reasonable yield. The sluggish reactivity we previously reported for nucleophilic addition to the C-4 ester of the 3-anthryl-isoxazole is exemplified by (1) slow hydrolysis rates (i.e., 60 h using refluxing aqueous LiOH)[9] and (2) the observation that *n*-BuLi deprotonates the C-5 methyl at −78 °C without noticeable addition to the C-4 ester.[19] The C-4 functionalization prior to the NOC stage is a definite advantage for the preparation of AIMs, and will be the subject of further study.

In summary, we have found that the *BHMS protocol* produced superior results, especially in sterically encumbered examples containing fused ring aromatic nitrile oxides and that these *conditions represent the methodology of choice* when standard enamine methodology fails. We will report on the chemical and pharmacological application of these novel isoxazoles in due course.

2. Experimental

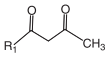
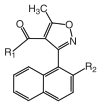
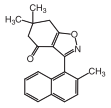
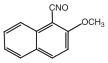
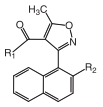
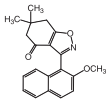
2.1. General

All reactions were performed under inert atmosphere. Purification was carried out by column chromatography. Chemicals were purchased from TCI or Aldrich Chemical Company, all commercial reagents are routinely examined for purity by NMR and TLC, and recrystallized or distilled as appropriate. Solvents were reagent grade. Melting points were determined in open capillary tubes on a Melt-Temp apparatus and are uncorrected. NMR spectra were obtained using either a Varian 400 MHz Unity Plus or a Varian NMR systems 500 MHz spectrometer, in deuteriochloroform unless otherwise noted. Infrared spectra were obtained on a thermo-Nicolet 633 FT-IR spectrometer.

Chemical shifts (δ) are reported using $CHCl_3$ (7.26 ppm for ^1H), $CDCl_3$ (77 ppm for ^{13}C) as references. High resolution mass spectra (HRMS) were obtained using a Micromass electrospray ionization (ESI)/time-of-flight mass spectrometry (LCTOF). Mass spectrometer samples were introduced using a Waters model 2690 separations module HPLC fitted with a C-18 reversed phase column (2.1 mm i.d., 5 cm). Elemental analyses for C, H, and N were performed by Midwest Microlab, Indianapolis, IN. All reactions were monitored by Thin Layer Chromatography (TLC). Purification was performed by flash column chromatography, and analytical samples were prepared by PTLC. Analytical LCMS (UV at 254 nm) and NMR were used to establish the purity of targeted compounds. All compounds that were evaluated in biochemical and biophysical assays had >95% purity as determined by ^1H NMR and LCMS NSC 728558 was prepared from **3k** as previously described.[8,9]

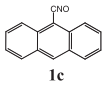
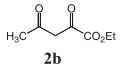
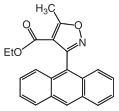
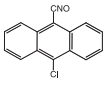
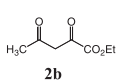
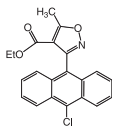
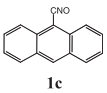
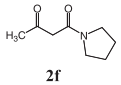
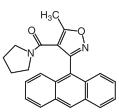
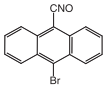
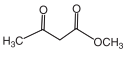
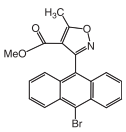
2.1.1. 1-(Pyrrolidin-1-yl)butane-1,3-dione.[18] Pyrolidine (0.539 g, 7.5 mmol) is dissolved in 10 mL of anhydrous DCM and diketene (0.925 g, 11.00 mmol) is added. The mixture is stirred at ambient temperature for 2.5 h, followed by removal of the solvent under

Table 1
Nitrile oxide cycloaddition synthesis of hindered 3-naphthyl-isoxazoles

Entry	Nitrile oxide	Dipolarophile	Conditions[a]	Product	Yield %
1	 **1a**, $R_2=CH_3$	 **2a**, $R_1=CH_3$	Et_3N/EtOH	 **3a**, $R_1=CH_3$; $R_2=CH_3$	40
2	**1a**, $R_2=CH_3$	**2b**, $R_1=OCH_2CH_3$	Na/EtOH	**3b**, $R_1=OCH_2CH_3$; $R_2=CH_3$	70
3	**1a**, $R_2=CH_3$	**2c**, $R_1=C_6H_5$	Et_3N	**3c**, $R_1=C_6H_5$; $R_2=CH_3$	40
4	**1a**, $R_2=CH_3$	 **2d**	Et_3N	 **3d**	71
5	 **1b**, $R_2=OCH_3$	**2a**, $R_1=CH_3$	Na/EtOH	 **3e**, $R_1=CH_3$; $R_2=OCH_3$	70
6	**1b**, $R_2=OCH_3$	**2e**, $R_1=OCH_3$	Na/MeOH 2.5 h	**3f**, $R_1=OCH_3$; $R_2=OCH_3$	60
7	**1b**, $R_2=OCH_3$	**2e**, $R_1=OCH_3$	Et_3N	**3f**, $R_1=OCH_3$; $R_2=OCH_3$	62
8	**1b**, $R_2=OCH_3$	**2b**, $R_1=OCH_2CH_3$	Na/EtOH	**3g**, $R_1=OCH_2CH_3$; $R_2=OCH_3$	80
9	**1b**, $R_2=OCH_3$	**2c**, $R_1=C_6H_5$	Na/EtOH	**3h**, $R_1=C_6H_5$; $R_2=OCH_3$	15
10	**1b**, $R_2=OCH_3$	**2c**, $R_1=C_6H_5$	Et_3N/EtOH	**3h**, $R_1=C_6H_5$; $R_2=OCH_3$	22
11	**1b**, $R_2=OCH_3$	**2c**, $R_1=C_6H_5$	NaO*i*-Pr	**3h**, $R_1=C_6H_5$; $R_2=OCH_3$	43
12	**1b**, $R_2=OCH_3$	 **2d**	Na/EtOH	 **3i**	42
13	**1b**, $R_2=OCH_3$	**2d**	Et_3N	**3i**	53
14	**1b**, $R_2=OCH_3$	**2d**	NaO*i*-Pr	**3i**	57

[a] The reaction was conducted in anoxic conditions.

Table 2
Nitrile oxide cycloaddition to formed hindered 3-anthracenyl-isoxazoles

Entry	Nitrile oxide	Dipolarophile	Conditions[a]	Product	Yield (%)
1	 **1c**	 **2b**	Na/EtOH	 **3j**	88–94
2	 **1d**	 **2b**	Na/EtOH	 **3k**	90–92
3	 **1c**	 **2f**	Na/EtOH	 **3l**	50
4	 **1e**	 **2e**	Na/MeOH	 **3m**	82
5	**1e**	**2e**	Et_3N	**3m**	72

[a] The reaction was conducted in anoxic conditions.

vacuum to afford (1.163 g, 99%) as red-brown liquid. ^1H NMR (500 MHz, $CDCl_3$): δ 3.46 (t, J=6.62 Hz, 2H), 3.45 (s, 2H), 3; ^{13}C NMR (125 MHz, $CDCl_3$): δ 202.6, 165.0, 88.8, 51.3, 47.2, 45.9, 30.4, 26.0, 24.4; IR cm^{-1}; mass calculated for $C_8H_{13}NO_2$ 155.0946, found m/z 156.1902 (M+1, 100%).

2.2. NOC: sodium enolate method

2.2.1. (3-(Anthracen-9-yl)-5-methylisoxazol-4-yl)(pyrrolidin-1-yl) methanone, ***3l****.* To a solution of sodium ethoxide, prepared from 0.199 g of Na in 44.2 mL of absolute EtOH, was added 1-(pyrrolidin-1-yl)butane-1,3-butane-1,3-dione (1.015 g, 6.54 mmol), and 9-anthrahydroximinoylchloride, (1.06, 4.105 mmol) successively and the resulting red solution was stirred under Ar atmosphere for 4 h at ambient temperature. The red-brown solution was extracted with ethyl acetate (4×20 mL), washed with DI water (2×50 mL) and brine (50 mL), and dried over anhydrous Na_2SO_4. Filtration and concentration afforded the desired product **3l** as red-brown oil (1.40 g, 95%), which was further purified by silica column flash chromatography (hexanes/EtOAc; 10:1), and PTLC to afford **3l** as off white solid (0.7375 g, 50%).

2.3. NOC: triethylamine method

2.3.1. Synthesis of 1-(5-methyl-3-(2-methylnaphthalene-1-yl)isoxazol-4-yl)ethanone, ***3a****.* To 2,4-pentanedione (0.42 g, 4.11 mmol) in absolute ethanol (21 mL) at ambient temperature was added

triethylamine (0.47 g, 4.584 mmol) followed by addition of nitrile oxide (0.6 g, 3.28 mmol). The temperature was raised to 53 °C and the mixture stirred under Ar atmosphere for 72 h. The pale yellow solution was extracted with chloroform (4×20 mL), washed with DI water (2×50 mL) and brine (50 mL), dried over anhydrous Na_2SO_4. Filtration, concentration and silica column flash chromatography (hexanes/EtOAc/DCM; 5:1:1) afforded the desired product **3a** (0.289 g, 40%).

2.4. Analytical data for 3-fused aryl isoxazole products

2.4.1. 1-(5-Methyl-3-(2-methylnaphthalene-1-yl)isoxazol-4-yl)ethanone, ***3a***. Oil, TLC SiO_2 hexane/DCM/EtOAc 4:1:1 R_f 0.77. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.80–7.75 (m, 2H), 7.38–7.32 (m, 4H), 2.76 (s, 3H), 2.26 (s, 3H), 1.56 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 192.3, 175.7, 159.6, 135.3, 132.3, 131.4, 129.5, 127.8, 126.8, 125.2, 124.0, 117.5, 28.6, 19.8, 13.6; HRMS calcd for $C_{17}H_{16}NO_2$ 266.1181; found: 266.1193.

2.4.2. Ethyl 3-(2-methylnaphthalen-1-yl)-5-methylisoxazole-4-carboxylate, ***3b***. Oil, TLC SiO_2 hexane/DCM/EtOAc 4:1:1 R_f 0.6. ^{1}H NMR ($CDCl_3$) δ 7.826 (d, 2H); 7.25–7.398 (m, 4H); 3.87 (q, 2H); 2.841 (s, 3H); 2.303 (s, 3H); 0.651 (t, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.7, 161.4, 160.7, 135.0, 132.5, 131.4, 128.9, 127.9, 127.7, 126.2, 124.8, 124.6, 124.5, 110.1, 59.9, 20.1, 13.2, 13.0. HRMS calcd for $C_{18}H_{18}NO_3$: 296.1287; found: 296.1297.

2.4.3. Phenyl (5-methyl-3-(2-methylnaphthalen-1-yl)isoxazol-4-yl) methanone, ***3c***. Oil, TLC SiO_2 hexane/DCM/EtOAc 4:1:1 R_f 0.79. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.65 (d, J=7.90, 1H), 7.60 (d, J=8.50 Hz, 1H), 7.55 (d, J=8.00 Hz, 1H), 7.37 (t, J=6.60 Hz, 1H), 7.33 (m, 3H), 7.16 (m, 2H), 6.94 (t, J=8.00 Hz, 2H), 3.38 (s, 3H), 2.64 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 189.8, 173.7, 171.0, 160.3, 137.1, 135.5, 132.4, 132.3, 131.4, 129.3, 129.2, 128.1, 128.0, 127.9, 127.8, 127.5, 125.0, 124.5, 124.4, 123.6, 117.8, 20.4, 14.0; HRMS calcd for $C_{22}H_{18}NO_2$: 328.1338; found: 328.1370.

2.4.4. 6,6-Dimethyl-3-(2-methylnaphthalen-1-yl)-6,7-dihydrobenzo [d]isoxazol-4(5H)-one ***3d***. Oil, TLC SiO_2 hexane/DCM/EtOAc 4:1:1 R_f 0.71. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.87 (d, J=8.3, 1H), 7.84 (d, J=8.3, 1H), 7.36–7.43 (m, 4H), 3.01 (s, 2H), 2.40 (d, J=8.3, 2H), 2.31 (s, 3H), 1.22 (s, 3H), 1.19 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 190.8, 180.9, 171.1, 157.9, 135.6, 132.3, 131.7, 129.7, 129.6, 128.4, 128.2, 128.1, 128.0, 125.2, 125.0, 124.5, 124.3, 122.9, 52.4, 37.0, 35.5, 28.6, 28.5, 28.1, 28.0, 21.0, 14.2. HRMS calcd for $C_{20}H_{20}NO_2$: 306.1494; found: 306.1516.

2.4.5. 1-(3-(2-Methoxynaphthalene-1-yl)-5-methylisoxazol-4-yl) ethanone, ***3e***. Oil, TLC SiO_2 hexane/DCM/EtOAc 4:1:1 R_f 0.53. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.01 (d, J=9.30 Hz, 1H), 7.84 (d, J=7.80 Hz, 1H), 7.48 (d, J=8.30 Hz, 1H), 7.44 (t, J=8.30 Hz, 1H), 7.39 (d, J=7.90 Hz, 1H), 7.36 (d, J=8.80 Hz, 1H), 3.90 (s, 3H), 2.81 (s, 3H), 1.76 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 193.2, 175.4, 157.7, 155.3, 133.3, 132.1, 128.7, 128.1, 127.8, 124.2, 123.9, 118.3, 112.6, 111.4, 56.4, 28.8, 14.0. HRMS calcd for M+H, $C_{17}H_{16}NO_3$ 282.1130; found: 282.1150 (M+1, 100%). Anal. Calcd for $C_{17}H_{15}NO_3$: C, 72.58; H, 5.37; N, 4.98; found: C, 71.81; H, 5.52; N, 4.56.

2.4.6. Methyl 3-(2-methoxynaphthalene-1-yl)-5-methylisoxazole-4-carboxylate, ***3f***. Oil, TLC SiO_2 hexane/DCM/EtOAc 4:1:1 R_f 0.56. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.96 (d, J=9.05 Hz, 1H), 7.82 (d, J=9.99 Hz, 1H), 7.55 (d, J=9.99 Hz, 1H), 7.40 (t, J=9.99 Hz, 1H), 7.35 (t, J=9.99 Hz, 2H), 3.86 (s, 3H), 3.54 (s, 3H), 2.81 (s, 3H); ^{13}C NMR (500 MHz, $CDCl_3$): δ 175.1, 162.1, 158.5, 155.3, 133.0, 131.3, 128.6, 127.9, 127.0, 124.0, 123.6, 112.6, 111.4, 110.4, 56.4, 51.3, 13.3; IR cm^{-1}; HRMS calcd for $C_{17}H_{16}NO_4$: 298.1079; found 298.1111. Anal. Calcd for $C_{17}H_{15}NO_4$: C, 68.68; H, 5.09; N, 4.71; found: C, 66.81; H, 5.00; N, 4.53.

2.4.7. Ethyl 3-(2-methoxynaphthalen-1-yl)-5-methylisoxazole-4-carboxylate, ***3g***. Yellow solid (1.8 g, 60%): mp 103–104 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.96 (d, J=8.99 Hz, 1H), 7.81 (d, J=8.07 Hz, 1H), 7.50 (d, J=8.55 Hz, 1H), 7.39 (dd, J=11.00, 6.84 Hz 1H), 7.34 (d, J=9.05 Hz, 2H), 3.96 (q, J=7.06 Hz, 2H), 3.88 (s, 3H), 2.82 (s, 3H), 0.79 (t, J=7.06 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 175.3, 161.9, 158.6, 155.3, 133.3, 131.3, 128.6, 127.9, 127.1, 124.1, 123.7, 112.7, 111.8, 110.6, 60.1, 56.5, 13.5, 13.3; ESI-MS calcd for $C_{18}H_{17}NO_4$+H m/z 312.1428 (M+1, 100%). Anal. Calcd for $C_{18}H_{17}NO_4$: C, 69.44; H, 5.50; N, 4.50. Found: C, 69.58; H, 5.47; N, 4.54.

2.4.8. Phenyl (3-(2-methoxynaphthalen-1-yl)-5-methylisoxazol-4-yl) methanone, ***3h***. Mp 141–142 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.06 (d, J=8.50, 1H), 7.73 (d, J=8.80 Hz, 2H), 7.51 (t, J=8.40 Hz, 1H), 7.44 (d, J=8.30 Hz, 2H), 7.36 (t, J=7.55 Hz, 1H), 6.96 (t, J=7.80 Hz, 2H), 6.90 (d, J=9.30 Hz, 1H), 3.61 (s, 3H), 2.69 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 189.8, 173.2, 157.8, 154.5, 137.1, 132.7, 132.4, 131.7, 128.7, 128.5, 128.0, 127.7, 127.5, 124.2, 117.6, 111.6, 78.1, 55.5, 29.7; ESI-MS for $C_{22}H_{17}NO_3$+H m/z 344.1008 (M+1, 100%).

2.4.9. 6,6-Dimethyl-3-(2-methoxynaphthalen-1-yl)-6,7-dihydrobenzo[d]isoxazol-4(5H)-one, ***3i***. Oil, TLC SiO_2 hexane/DCM/EtOAc 4:1:1 R_f 0.46. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.96 (d, J=9.1, 1H), δ 7.81 (d, J=7.8 Hz, 1H), 7.69 (d, J=8.6 Hz, 1H), 7.43 (dt, J=9.8 Hz, 1.5 Hz, 1H), 7.36 (dd, J=6.8 Hz, 1.3 Hz, 1H), 7.34 (d, J=9.3 Hz, 2H), 3.85 (s, 3H), 2.90 (s, 2H), 2.36 (q, J=14.7 Hz, 2H), 1.17 (s, 3H), 1.14 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 190.7, 180.2, 155.7, 155.4, 132.6, 131.7, 131.6, 128.6, 128.1, 128.0, 127.9, 127.8, 127.6, 127.2, 126.9, 123.9, 123.7, 123.4, 115.2, 112.9, 112.8, 112.6, 109.9, 56.4, 56.2, 52.3, 36.7, 35.1, 28.3, 28.0. HRMS calcd for $C_{20}H_{20}NO_3$ 322.1443; found: 322.1458.

2.4.10. Ethyl 3-(anthracen-9-yl)-5-methylisoxazole-4-carboxylate, ***3j***. Mp 121–122 °C; TLC SiO_2 hexane/EtOAc 10:1, R_f 0.30. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.58 (s, 1H), 8.05 (d, J=8.31 Hz, 2H), 7.65 (d, J=8.55 Hz, 2H), 7.40–7.49 (m, 4H), 3.71 (q, J=7.21 Hz, 2H), 2.93 (s, 3H), 0.32 (t, J=7.10 Hz, 3H); ^{13}C NMR (500 MHz, $CDCl_3$): 176.2, 161.5, 160.5, 131.0, 130.8, 128.7, 128.5, 126.3, 125.4, 125.2, 122.7, 111.4, 60.1, 13.5, 12.8, spectral data are in accord with those reported previously.[16a] ESI-MS for $C_{21}H_{17}NO_3$+H m/z 332.1441 (M+1, 100%).

2.4.11. Ethyl 3-(10-chloroanthracen-9-yl)-5-methylisoxazole-4-carboxylate, ***3k***. Mp 123–124 °C, lit. Mp 119–120.[16b] TLC SiO_2 hexane/EtOAc 10:1, R_f 0.28. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.59 (d, J=8.80 Hz, 2H), 7.53–7.60 (m, 4H), 7.43 (d, J=8.80 Hz, 2H), 3.72 (q, J=7.1 Hz, 2H), 2.93 (s, 3H), 0.39 (t, J=7.1 Hz, 3H); ^{13}C NMR (500 MHz, $CDCl_3$): 177.3, 161.3, 161.2, 134.1, 131.1, 128.3, 126.7, 125.9, 125.1, 122.6, 111.5, 60.2, 13.2; ESI-MS for $C_{21}H_{16}ClNO_3$+H, m/z 366.0886 (M+1, 95%).

2.4.12. [3-(Anthracen-9-yl)-5-methylisoxazol-4-yl](pyrrolidin-1-yl) methanone, ***3l***. Mp 194–195 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.54 (s, 1H), 7.99 (d, J=9.29 Hz, 2H), 7.95 (d, J=10.0 Hz, 2H), 7.48–7.45 (m, 4H), 3.10 (t, J=6.87 Hz, 2H), 2.73 (s, 3H), 2.60 (t, J=6.61 Hz, 2H), 1.42 (pentet, J=6.85 Hz, 2H), 1.29 (pentet, J=6.60 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.1, 161.3, 158.3, 131.0, 130.5, 129.4, 128.5, 126.9, 126.7, 125.8, 125.3, 125.0, 121.9, 116.5, 47.7, 45.3, 25.6, 23.7, 12.4; ESI-MS for $C_{23}H_{20}N_2O_2$+H, m/z 357.0757 [M+1, (100)]. Anal. Calcd for $C_{23}H_{20}N_2O_2$: C, 77.51; H, 5.66; N, 7.86. Found: C, 77.37; H, 5.45; N, 7.99.

2.4.13. Methyl 3-(10-bromoanthracen-9-yl)-5-methylisoxazole-4-carboxylate, ***3m***. R_f 0.45 hexane/EtOAc/DCM 4:1:1. ^{1}H NMR (500 MHz, $CDCl_3$): 8.63–8.60 (d, 2H), 7.62–7.59 (m, 4H), 7.47–7.43 (m, 2H), 3.32 (s, 3H), 2.93 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): 176.08, 163.32, 161.53, 131.12, 129.90, 127.96, 126.88, 126.39, 125.60,

123.12, 51.35, 13.42. HRMS calcd for M+H $C_{20}H_{14}NO_3{}^{79}Br + H$, m/z 396.0227; found: 396.0235.

2.5. Laser Scanning Cytometry methods

SNB-19 human glioblastoma cells (American Type Cell Culture Cat No. CRL-2266) were plated at a density of 2000 cells/mL on cover slips in RPMI medium (with L-glutamine and penicillin/streptomycin and supplemented with 10% fetal bovine serum). Cell culture medium and supplements were obtained from VWR (West Chester, PA). The cells were incubated at 37 °C under a humidified atmosphere containing 5% CO_2 and allowed to adhere. Medium was aspirated and replaced with 1 μM NSC 728558 for 24-h exposure. Drug was removed and cells were washed twice with phosphate buffered saline (PBS). Cells were fixed in 4% paraformaldehye (15 min, 21 °C) and washed once with PBS. Following two additional washes, slides were inverted to microscope slides and sealed using FluorSave Reagent (Calbiochem). Images were generated from scans from a CompuCyte iCys Laser Scanning Cytometer. (CompuCyte, Westwood, MA). Cells were scanned with a 405 nm 30 mW Diode laser. Fluorescent signals were measured in photomultiplier detectors following a 440/30 Band-pass (BP) filter to detect NSC 728558 presence. Light absorption was also measured to produce a differential interference contrast (DIC)-like image for cell morphology. Each signal was given a pseudo-color and overlaid to produce images.

Acknowledgements

The authors thank the NIH for grants R21NS067466, P20RR017670 and P30NS055022 for financial support of this work.

Supplementary data

Representative HPLC trace for **3m**. Characterization data for by-products **7** and **8**, representative spectra for products **3**. Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.tet.2012.09.084.

References and notes

1. (a) Zhu, S.; Shi, S.; Gerritz, S. W. *Tetrahedron Lett.* **2011**, *52*, 4001–4004 Refs. 1–4of this paper reviews recent bioactive isoxazoles in the patent literature; (b) Nakamura, M.; Kurihara, H.; Suzuki, G.; Mitsuya, M.; Ohkubo, M.; Ohta, H. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 726–729.
2. The Kurth group has provided a useful catalog of the bioactive isoxazole literature in their NOC synthesis papers over the years, the following are illustrative examples: (a) Yu, G. J.; Yang, B.; Verkman, A. S.; Kurth, M. J. *Synlett* **2010**, 1063–1066; (b) Meng, L.; Lorsbach, B. A.; Sparks, T. C.; Fettinger, J. C.; Kurth, M. J. *J. Comb. Chem.* **2010**, *12*, 129–136; (c) Butler, J. D.; Donald, M. B.; Ding, Z.; Fettinger, J. C.; Kurth, M. J. *Tetrahedron Lett.* **2009**, *50*, 5110–5112; (d) Choung, W.; Lorsbach, B. A.; Sparks, T. C.; Ruiz, J. M.; Kurth, M. J. *Synlett* **2008**, 3036–3040; (e) Quan, C.; Kurth, M. *J. Org. Chem.* **2004**, *69*, 1470–1474; (f) Hwang, S. H.; Kurth, M. J. *J. Org. Chem.* **2002**, *67*, 6564–6567.
3. In the course of their studies on glutamate receptor and transporter ligand pharmacology, the Krogsgaard-Larsen group has greatly expanded the tool box for isoxazole synthesis:*Glutamate and GABA Receptors and Transporters, Structure Function and Pharmacology*; Egebjerg, J., Schousboe, A., Krogsgaard-Larsen, P., Eds.; Taylor and Francis: New York, NY, 2002.
4. Pinho e Melo, T. M. V. D. *Curr. Org. Chem.* **2005**, *9*, 925–958.
5. Trogu, E.; Cecchi, L.; De Sarlo, F.; Guideri, L.; Ponticelli, F.; Machetti, F. *Eur. J. Org. Chem.* **2009**, 5971–5978 and cited references.
6. Bode, J. W.; Hachisu, Y.; Matsuura, T.; Suzuki, K. *Org. Lett.* **2003**, *5*, 391–394.
7. Bode, J. W.; Hachisu, Y.; Matsuura, T.; Suzuki, K. *Tetrahedron Lett.* **2003**, *44*, 3555–3558.
8. Han, X.; Li, C.; Mosher, M. D.; Rider, K. C.; Zhou, P.; Crawford, R. L.; Fusco, W.; Paszczynski, A.; Natale, N. R. *Bioorg. Med. Chem.* **2009**, *17*, 1671–1680.
9. Gajewski, M. P.; Beall, H.; Schnieder, M.; Stranahan, S. M.; Mosher, M. D.; Rider, K. C.; Natale, N. R. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4067–4069.
10. (a) Veiseh, M.; Gabikian, P.; Bahrami, S.-B.; Veiseh, O.; Zhang, M.; Hackman, R. C.; Ravanpay, A. C.; Stroud, M. R.; Kusuma, Y.; Hansen, S. J.; Kwok, D.; Munoz, N. M.; Sze, R. W.; Grady, W. M.; Greenberg, N. M.; Ellenbogen, R. G.; Olson, J. M. *Cancer Res.* **2007**, *67*, 6882–6888; (b) Smeltzer, C.; Cannon, M. J.; Pinson, P.; Munger, J. S.; West, F. G.; Grissom, C. B. *Org. Lett.* **2001**, *3*, 799–801; (c) McGreevy, J. M.; Cannon, M.; Grissom, C. B. *J. Surg. Res.* **2003**, *111*, 38–44.
11. Feuer, H. In *Nitrile Oxides, Nitrones and Nitronates in Organic Synthesis*; John Wiley & Sons: Hoboken, New Jersey, 2008.
12. (a) Stork, G.; McMurry, J. E. *J. Am. Chem. Soc.* **1967**, *89*, 5461–5462; (b) McMurry, J. E. *Organic Syntheses*; 1988; Collect. Vol. No. VI 582–594.
13. Natale, N. R.; McKenna, J. I.; Niou, C.-S.; Borth, M.; Hope, H. *J. Org. Chem.* **1985**, *50*, 5660–5666.
14. Zamponi, G.; Stotz, S. C.; Staples, R. J.; Rogers, T. A.; Nelson, J. K.; Hulubei, V.; Blumenfeld, A.; Natale, N. R. *J. Med. Chem.* **2003**, *46*, 87–96 and cited references.
15. Bowles, K. D.; Quincy, D. A.; Mallet, B.; McKenna, J. I.; Natale, N. R. *J. Chem. Educ.* **1985**, *62*, 1118–1120.
16. (a) Mosher, M. D.; Natale, N. R. *J. Heterocycl. Chem.* **1995**, *32*, 779–781; (b) Han, X.; Twamley, B.; Natale, N. R. *J. Heterocycl. Chem.* **2003**, *40*, 539–545.
17. Grundmann, C.; Dean, J. M. *J. Org. Chem.* **1965**, *30*, 2809–2812.
18. (a) Beger, J.; Thielemann, C. *J. Prakt. Chem./Chem.-Ztg.* **1981**, *323*, 337–344; (b) Fujisawa, T.; Sato, T.; Takeuchi, M. *Chem. Lett.* **1982**, *1*, 71–74.
19. Han, X.; Li, C.; Rider, K. C.; Blumenfeld, A.; Twamley, B.; Natale, N. R. *Tetrahedron Lett.* **2002**, *43*, 7673–7677.
20. (a) Renzi, G.; Dal Piaz, V. *Gazz. Chim. Ital.* **1965**, *95*, 1478–1491; (b) Renzi, G.; Pinzauti, S. *IL Farmaco Ed. Sci.* **1969**, *24*, 885–892.
21. (a) Liu, B.; Liu, G.; Xin, Z.; Serby, M. D.; Zhao, H.; Schaefer, V. G.; Falls, D.; Kaszubska, W.; Collins, C. A.; Sham, H. L. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5223–5226; (b) Xin, Z.; Zhao, H.; Serby, M. D.; Liu, B.; Schaefer, V. G.; Falls, D. H.; Kaszubska, W.; Colins, C. A.; Sham, H. L.; Liu, G. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1201–1204.
22. Butler, J. D.; Coffman, K. C.; Ziebart, K. T.; Toney, M. D.; Kurth, M. J. *Chem.—Eur. J.* **2010**, *16*, 9002–9005.