Research Article

Received: 31 May 2017 Revised: 25 August 2017 Accepted article published: 4 September 2017 Published online in Wiley Online Library: 23 October 2017

(wileyonlinelibrary.com) DOI 10.1002/ps.4722

Synthesis and structure–activity relationships of carbohydrazides and 1,3,4-oxadiazole derivatives bearing an imidazolidine moiety against the yellow fever and dengue vector, *Aedes aegypti*

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Abstract

BACKGROUND: 1,3,4-Oxadiazole and imidazolidine rings are important heterocyclic compounds exhibiting a variety of biological activities. In this study, novel compounds with oxadiazole and imidazolidine rings were synthesized from *3-(methylsulfonyl)-2-oxoimidazolidine-1-carbonyl chloride* and screened for insecticidal activities. The proposed structures of the 17 synthesized compounds were confirmed using elemental analysis, infrared (IR), proton nuclear magnetic resonance (^{1}H-NMR), and mass spectroscopy.

RESULTS: None of the compounds showed larvicidal activity at the tested concentrations against first-instar *Aedes aegypti* larvae. However, nine compounds exhibited promising adulticidal activity, with mortality rates of ≥80% at 5 μg per mosquito. Further dose–response bioassays were undertaken to determine median lethal dose (LD_{50}) values. Compounds 1, 2b, 2c, 2d, 2 g, 3b, 3c, 3 g, and 3 h were effective, with typical LD_{50} values of about 5 − 10 μg per mosquito against female *Ae. aegypti*. Compounds 2c (bearing a nitro group on the aromatic ring; $LD_{50} = 2.80 \pm 0.54$ μg per mosquito) and 3 h (double halogen groups at 2,4 position on the phenyl ring; $LD_{50} = 2.80 \pm 0.54$ μg per mosquito) were the most promising compounds.

CONCLUSION: Preliminary mode of action studies failed to show consistent evidence of either neurotoxic or mitochondria-directed effects. Further chemical synthesis within this series may lead to the development of new effective insecticides.

Keywords: insecticide; mosquitocidal activity; mosquito control; carbohydrazide and amide; mitochondria

1 INTRODUCTION

Mosquito-borne diseases are a global problem and continued threat to public health. Malaria is still the most devastating parasitic disease that is transmitted by *Anopholes* mosquitoes.[1] Zika virus is an *Aedes* mosquito-borne flavivirus that has rapidly spread throughout South America, Central America, and the Caribbean.[2] Because vaccines or other specific treatments are not available for Zika virus infection,[3] chemical insecticides remain a major method for disease reduction by mosquito control. However, the emergence of resistance to the majority of existing compounds has prompted a continued demand for new insecticides.[4,5] Most current adulticides act via inhibition of acetylcholinesterase enzymes or blocking inactivation of voltage-sensitive sodium channels.[1]

Imidazolidine rings are heterocyclic moieties present in compounds that show a variety of biological activities.[6,7] Imidacloprid [*N-(1-((6-chloropyridine-3-yl)methyl)-4,5-dihydro-1H-imidazol-2-yl) nitramide*] was developed as the first neonicotinoid insecticide

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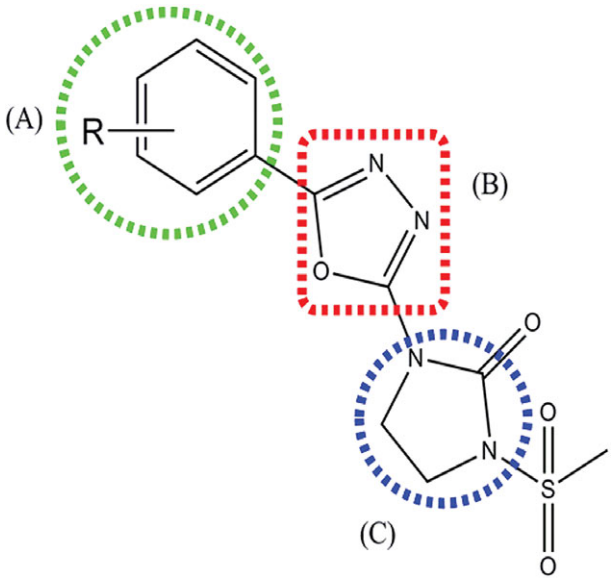


Scheme 1. General structure of synthesized compounds. (A) aromatic ring, (B) oxadiazole ring and (C) imidazolidinone ring.

that contained pyridine and imidazoline rings. Neonicotinoids are neuroactive insecticides that act on the nicotinic acetylcholine receptor (nAChR) at the postsynaptic membrane in insects. [8,9]

Chemical structures of compounds were envisioned consisting of three parts: an aromatic ring (A), oxadiazole ring (B) and imidazolidinone ring (C). Further, a carbonyl group with a hydrazide moiety can be used instead of the oxadiazole ring.[10] The 1,3,4-oxadiazole ring is a useful heterocyclic moiety in medicinal chemistry and substituted 1,3,4-oxadiazole ring compounds exhibit different biological activities such as antibacterial,[11] herbicidal,[12] insecticidal,[13] antiviral,[14] antitumoral[15] and anti-inflammatory activities.[16] In addition to a broad spectrum of biological activities, 1,3,4-oxadiazole rings are bioisosteres of amide and ester functional groups, and it is reported that these rings can easily participate in hydrogen-bonding interactions with various different receptors.[17]

In our previous study, we synthesized a series of 3-acetyl-2,5-disubstituted-2,3-dihydro-1,3,4-oxadiazoles. Out of the 17 compounds tested, only 3-acetyl-5-(4-fluorophenyl)-2-phenyl-2,3-dihydro-1,3,4-oxadiazole and 3-acetyl-5-(4-fluoro phenyl)-2-(4-bromophenyl)-2,3-dihydro-1,3,4-oxadiazole possessed larvicidal activity against first-instar *Aedes aegypti* L. with LC_{50} values (concentration that will kill 50% of the larval mosquitoes) of 24.1 and 30.9 ppm, respectively.[18] The recent outbreak of Zika virus infection has accelerated the search for novel mosquito control agents[19] and both larval and adult mosquito control tools are important in an integrated vector management strategy.[20] For continuation of our research aimed at the discovery of novel insecticidal agents, 17 novel compounds containing oxadiazole and imidazolidine rings were synthesized and evaluated for larvicidal and adulticidal activity against *Ae. aegypti*.

2 MATERIALS AND METHODS

2.1 Chemistry

All chemical reagents and solvents were procured from Sigma-Aldrich (St. Louis, MO, USA), and Merck (Darmstadt, Germany), except for Carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone (FCCP), which was acquired from Abcam (Cambridge, MA, USA). The homogeneity and purity of the compounds were checked by thin layer chromatography (TLC), performed on commercially available silica gel (Kieselgel 60, F254, Merck KGaA, 64271, Darmstadt, Germany) coated aluminum sheets (TLC Silica gel 60G F_{254} 25 Glass plates 20 × 20 cm Darmstadt, Germany) using methanol:chloroform (60:40) as the solvent system. Visualization on TLC was performed using both ultraviolet (UV) light ($\lambda = 254$ nm) and iodine indicators. The purities of the synthesized compounds were checked by reversed phase high-performance liquid chromatography (HPLC) (on a Chromasil C_{18} 3.6 × 150 mm column, Hichrom limited, Theale, UK) using acetonitrile and water (50:50 v/v) as the eluent. Melting points were determined using Schmelzpunktbestimmer SMP II (Gottfried-Keller-Weg, Überlingen, Germany). Infrared (IR) spectra were recorded with an FTIR-8400S (Shimadzu, Tokyo, Japan). Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on an Avance 400 MHz (Bruker BioSpin Corporation, Fremont, CA, USA) in dotero-dimetylsulfoxide (DMSO-d_6) using tetramethylsilane (TMS) as the internal reference. Chemical shifts (δ) were expressed in parts per million relative to TMS and the following abbreviations were used to describe the peak patterns when appropriate: s, singlet; d, doublet; t, triplet; m, multiplet. Elemental analysis [for carbon (C), hydrogen (H) and nitrogen (N)] was performed on a CHNS-Thermo Scientific Flash 2000 (Thermo Fisher Scientific, Waltham, MA, USA).

2.2 General procedure for the synthesis of the hydrazide derivatives

3-(Methylsulfonyl)-2-oxoimidazolidine-1-carbohydrazide (**1**).

CAS Number:64341-28-2.

3-(Methylsulfonyl)-2-oxoimidazolidine-1-carbonyl chloride (1.74 mmol) was dissolved in ethanol on a magnetic stirrer. Then, hydrazine monohydrate (1 mL) was added and the mixture stirred at room temperature for 8 h. The mixture was filtered and washed with ethanol.[21]

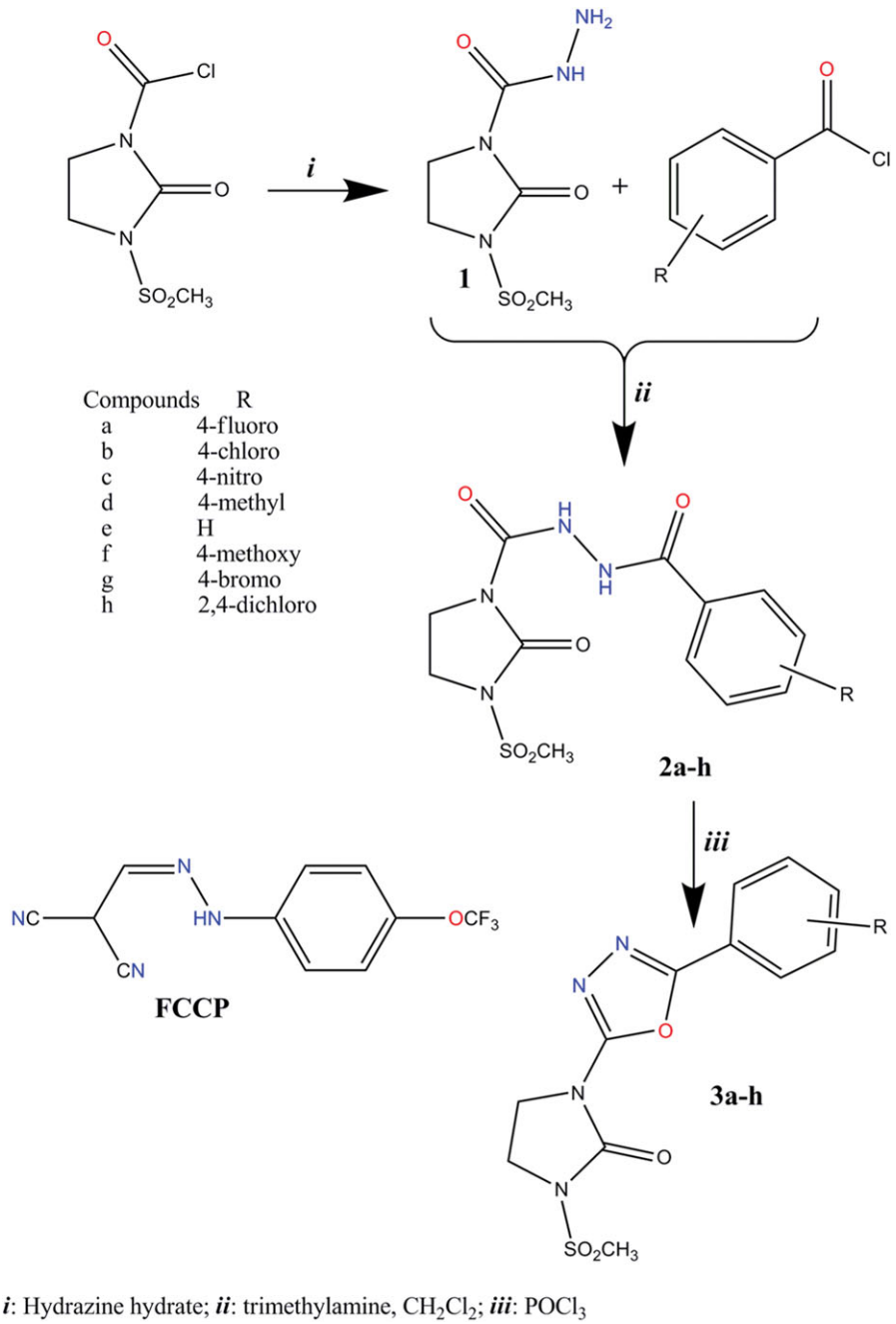
Data for **1**. White solid, yield 60%, melting point (mp) 222 – 223°C. IR υ_{max} (maximum absorption wave number) (cm^{-1}): 3566, 3358, 3020, 2997, 2918, 1685, 1508, 1477, 1288. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 3.37 (s, 3H, SO_2CH_3), 3.72-3.85 (m, 4H, $-CH_2-$), 4.85 (s, 2H, $-NH_2$), 9.36 (s, 1H,-NH-). For $C_5H_{10}N_4O_4S$ [molecular weight (M.W.): 222.22 g/mol], calculated (calcd): C: 27.02 H: 4.54 N: 25.21, found: C 27.02 H: 4.54 N: 25.21.

2.3 General procedure for the synthesis of the carbohydrazide derivatives (2a – h)

To a solution of hydrazide (1 mmol) **(1)** and trimethylamine (2 mmol) in dry CH_2Cl_2 (5 mL), a solution of previously prepared benzoyl chloride (1 mmol) was added dropwise at room temperature. The reaction mixture was stirred on a magnetic stirrer for 3 h. Then the precipitate was washed with distilled water and filtered. The purity of compounds was checked with TLC.[22]

Data for N′-(4-fluorobenzoyl)-3-(methylsulfonyl)-2-oxoimidazolidine-1-carbohydrazide (**2a**). White solid, yield 70%, mp 271-273°C. IR υ_{max} (cm^{-1}): 3371, 3321, 3010, 2928, 1695, 1473, 1280, 812. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 3.37 (s, 3H, $-SO_2CH_3$), 3.77-4.01 (m, 4H, $-CH_2-$), 7.42-8.40 (m, 4H, Ar-H), 9.01 and 10.47 (s, 2H,-NH-). For $C_{12}H_{13}FN_4O_5S$ (M.W.: 344.32 g/mol), calcd: C: 41.86 H: 3.81 N: 16.27, found: C: 41.66 H: 3.79 N: 16.20.

Data for N′-(4-chlorobenzoyl)-3-(methylsulfonyl)-2-oxoimidazolidine-1-carbohydrazide (**2b**). White solid, yield 70%, mp 248-250°C. IR υ_{max} (cm^{-1}): 3367, 3282, 3026, 2993, 2933, 1689, 1479, 1280, 848. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 3.37 (s, 3H, $-SO_2CH_3$), 3.75-3.92



Scheme 2. The synthesis route of the target compounds and the commercially available mitochondrial uncoupler FCCP.

(m, 4H, -CH_2-), 7.58-7.90 (m, 4H, Ar-H), 9.36 and 10.67 (s, 2H, -NH-). For $C_{12}H_{13}ClN_4O_5S$ (M.W.: 360.77 g/mol^{-1}), calcd: C: 39.95 H: 3.63 N: 15.53, found: C: 40.01 H: 3.60 N: 15.55.

Data for 3-(methylsulfonyl)-N′-(4-nitrobenzoyl)-2-oxoimidazolidine-1-carbohydrazide (**2c**). Pale yellow, yield 60%, mp 286-288°C. IR υ_{max} (cm^{-1}): 3302, 3020, 2993, 2937, 1687, 1479, 1251, 800. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 3.37 (s, 3H, -SO_2CH_3), 3.73-3.92 (m, 4H, -CH_2-), 7.20-7.33 (d, 4H, Ar-H), 9.02 and 9.24 (s, 2H, NH). For $C_{12}H_{13}N_5O_7S$ (M.W.: 371.33 g/mol^{-1}), calcd: C: 38.81 H: 3.53 N: 18.86, found: C: 38.84 H: 3.54 N: 18.80.

Data for N′-(4-methylbenzoyl)-3-(methylsulfonyl)-2-oxoimidazolidine-1-carbohydrazide (**2d**). White solid, yield 65%, mp 245-246°C. IR υ_{max} (cm^{-1}): 3373, 3300, 3032, 2982, 2924, 1695, 1473, 1253, 802. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 2.49-2.51(s, 3H, -CH_3) 3.39 (s, 3H, -SO_2CH_3), 3.74-3.89 (m, 4H, -CH_2-), 7.28-7.84 (m, 4H, Ar-H), 9.41 (s, 2H, -NH-). For $C_{13}H_{16}N_4O_5S$ (M.W.: 340.35 g/mol^{-1}), calcd: C: 45.88 H: 4.74 N: 16.46, found: C: 46.01 H: 4.72 N: 16.50.

Data for N′-benzoyl-3-(methylsulfonyl)-2-oxoimidazolidine-1-carbohydrazide (**2e**). White solid, yield 70%, mp 240-242°C. IR υ_{max} (cm^{-1}): 3371, 3327, 3024, 2989, 2935, 1693, 1473, 1251, 802. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 3.39 (s, 3H, -SO_2CH_3), 3.74-3.90 (m, 4H, -CH_2-), 7.51-7.89 (m, 5H, Ar-H), 9.36 and 9.44 (s, 2H, -NH-). For $C_{12}H_{14}N_4O_5S$ (M.W.: 326.33 g/mol^{-1}), calcd: C: 44.17 H: 4.32 N: 17.17, found: C: 44.15 H: 4.30 N: 17.20.

Data for N′-(4-methoxybenzoyl)-3-(methylsulfonyl)-2-oxoimidazolidine-1-carbohydrazide (**2f**). Pale yellow, yield 60%, mp 261-263°C. IR υ_{max} (cm^{-1}): 3281, 3227, 3009, 2922, 2841, 1703, 1489, 1249, 898. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 3.39 (s, 3H,

Table 1. Percentage mortality at 24 h using the ORL strain of adult *Aedes aegypti* ($n = 3$)

Compound	% mortality at 5 µg per mosquito (mean ± SE)	LD_{50} (µg per mosquito) (mean ± SE)[a]	95% CI	R^2
1	83.3 ± 15.3	3.08 ± 0.27	2.33 – 3.75	0.858
2a	53.3 ± 25.2	-	-	-
2b	96.7 ± 5.8	3.61 ± 0.20	3.11 – 4.13	0.912
2c	80 ± 10	2.80 ± 0.54	1.45 – 4.77	0.655
2d	80 ± 20	3.10 ± 0.49	2.02 – 4.88	0.788
2e	70 ± 30	-	-	-
2f	66.7 ± 15.3	-	-	-
2 g	83.3 ± 5.8	3.59 ± 0.70	2.20 – 6.44	0.715
2 h	70 ± 10	-	-	-
3a	70 ± 34.6	-	-	-
3b	83.3 ± 20.8	3.49 ± 0.60	2.15 – 5.80	0.702
3c	86.7 ± 15.3	4.96 ± 1.21	3.26 – n.d.	0.631
3d	50 ± 10	-	-	-
3e	73.3 ± 15.3	-	-	-
3f	36.7 ± 20.8	-	-	-
3 g	86.7 ± 11.5	3.55 ± 0.24	3.00 – 4.17	0.902
3 h	86.7 ± 5.8	2.39 ± 0.15	2.03 – 2.72	0.962

[a] After the primary screening of the compounds, compounds showing mortality ≥80% were further tested in the adult topical LD_{50} dose–response bioassays.

$-SO_2CH_3$), 3.74-3.90 (m, 4H, $-CH_2-$), 4.33 (s, 3H, OCH_3), 7.00-7.90 (m, 4H, Ar-H), 9.37 (s, 2H, NH). For $C_{13}H_{16}N_4O_6S$ (M.W.: 356.35 g/mol^{-1}), calcd: C: 43.82 H: 4.53 N: 15.72, found: C: 43.91 H: 4.50 N: 15.80.

Data for N′-(4-bromobenzoyl)-3-(methylsulfonyl)-2-oxoimidazolidine-1-carbohydrazide (**2 g**). White solid, yield 65%, mp 290-291°C. IR υ_{max} (cm^{-1}): 3371, 3026, 2991, 2929, 1697, 1473, 1251, 898. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 3.39 (s, 3H, $-SO_2CH_3$), 3.77-3.92 (m, 4H, $-CH_2-$), 7.72-7.83 (m, 4H, Ar-H), 9.36 and 9.47 (s, 2H, -NH-). For $C_{12}H_{13}BrN_4O_5S$ (M.W.: 405.22 g/mol^{-1}), calcd: C: 35.57 H: 3.23 N: 13.83, found: C: 35.52 H: 3.26 N: 13.81.

Data for N′-(2,4-dichlorobenzoyl)-3-(methylsulfonyl)-2-oxoimidazolidine-1-carbohydrazide (**2 h**). White solid, yield 55%, mp 287-288°C. IR υ_{max} (cm^{-1}): 3367, 3302, 3020, 2993, 2929, 1687, 1479, 1251, 891. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 3.39 (s, 3H, $-SO_2CH_3$), 3.77-3.89 (m, 4H, $-CH_2-$), 7.46-7.56 (m, 3H, Ar-H), 9.36 and 9.57 (s, 2H, -NH-). For $C_{12}H_{12}Cl_2N_4O_5S$ (M.W.: 395.22 g/mol^{-1}), calcd: C: 36.47 H: 3.06 N: 14.18, found: C: 36.47 H: 3.01 N: 14.20.

2.4 General procedure for the synthesis of the oxadiazole derivatives (3a – h)

Carbohydrazide (1 g) was placed into a reaction flask and 15 mL of $POCl_3$ was added. The reaction mixture was refluxed for 6 h. Then the mixture was cooled to room temperature and poured into an ice – water mixture. The precipitate was filtered, washed and dried.[23]

Data for 1-[5-(4-fluorophenyl)-1,3,4-oxadiazol-2-yl]-3-(methylsulfonyl)imidazolidin-2-one (**3a**). White solid, yield 50%, mp 251-253°C. IR υ_{max} (cm^{-1}): 3375, 3080, 1683, 1593, 1475, 1234, 1097, 846. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 3.39 (s, 3H, $-SO_2CH_3$), 3.75-4.11 (m, 4H, $-CH_2-$), 7.44-8.02 (m, 4H, Ar-H). For $C_{12}H_{11}FN_4O_4S$ (M.W.: 326.30 g/mol^{-1}), calcd: C: 44.17 H: 3.40 N: 17.17, found: C: 44.24 H: 3.43 N: 17.10.

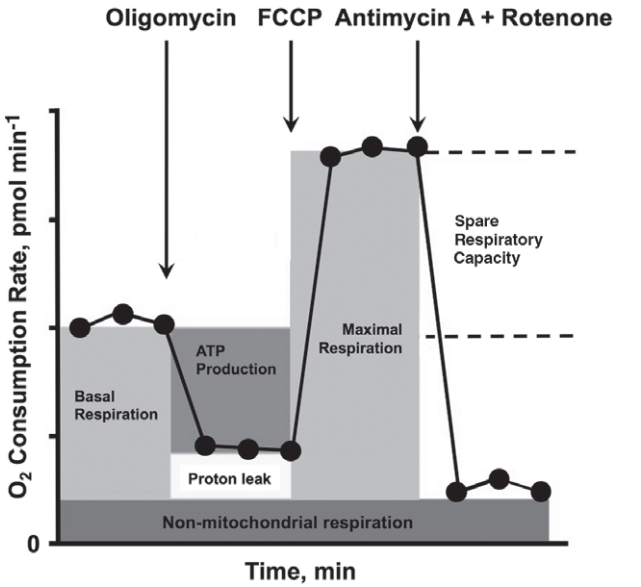


Figure 1. Test procedure for analysis of toxicant effects on parameters of mitochondrial function (redrawn from the Agilent Seahorse XF Cell MitoStress Test Kit User Guide, p. 6). Basal respiration reflects oxygen consumption of the cells prior to treatment with probes having defined effects on mitochondria. Application of oligomycin blocks complex IV ATP synthase to indicate respiration as a result of cellular ATP production. The uncoupling action of FCCP was used to estimate the maximal respiration rate, which then allowed calculation of spare respiratory capacity above basal rate. Final application of antimycin A + rotenone application blocks complexes III and I, respectively, and all respiration except non-mitochondrial processes. Proton leak (basal respiration not related to ATP production) and acute toxicity (lack of response to treatment) can then be calculated (Figure 2).

Data for 1-[5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl]-3-(methylsulfonyl)imidazolidin-2-one (**3b**). White solid, yield 50%, mp 276-278°C. IR υ_{max} (cm^{-1}): 3010, 2929, 1683, 1550, 1475, 1253, 809. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 3.39 (s, 3H, $-SO_2CH_3$), 3.74-4.08 (m, 4H, $-CH_2-$), 7.68-7.96 (m, 4H, Ar-H). For $C_{12}H_{11}ClN_4O_5S$ (M.W.: 342.76 g/mol^{-1}), calcd: C: 42.05 H: 3.23 N: 16.35, found: C: 42.10 H: 3.26 N: 16.40.

Data for 1-(methylsulfonyl)-3-[5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl]imidazolidin-2-one (**3c**). Pale yellow, yield 40%, mp 261-263°C. IR υ_{max} (cm^{-1}): 3024, 2931, 1685, 1555, 1471, 1230, 856. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 3.39 (s, 3H, $-SO_2CH_3$), 3.75-4.12 (m, 4H, $-CH_2-$), 8.16-8.47 (m, 4H, Ar-H). For $C_{12}H_{11}N_5O_6S$ (M.W.: 353.31 g/mol^{-1}), calcd: C: 40.79 H: 3.14 N: 19.82, found: C: 40.74 H: 3.10 N: 19.90.

Data for 1-[5-(4-methylphenyl)-1,3,4-oxadiazol-2-yl]-3-(methylsulfonyl)imidazolidin-2-one (**3d**). White solid, yield 40%, mp 241-242°C. IR υ_{max} (cm^{-1}): 3026, 2929, 1683, 1550, 1477, 1251, 800. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 2.50 (s, 3H, $-CH_3$), 3.39 (s, 3H, $-SO_2CH_3$), 3.74-4.07 (m, 4H, $-CH_2-$), 7.40-7.84 (m, 4H, Ar-H). For $C_{13}H_{14}N_4O_4S$ (M.W.: 322.34 g/mol^{-1}), calcd: C: 48.44 H: 4.38 N: 17.38, found: C: 48.48 H: 4.34 N: 17.40.

Data for 1-(methylsulfonyl)-3-(5-phenyl-1,3,4-oxadiazol-2-yl)imidazolidin-2-one (**3e**). White solid, yield 50%, mp 249-250°C. IR υ_{max} (cm^{-1}): 3024, 2929, 1683, 1548, 1469, 1249, 891. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 3.39 (s, 3H, $-SO_2CH_3$), 3.76-3.90 (m, 4H, $-CH_2-$), 7.50-8.24 (m, 5H, Ar-H). For $C_{12}H_{12}N_4O_4S$ (M.W.: 308.31 g/mol^{-1}), calcd: C: 46.75 H: 3.92 N: 18.17, found: C: 46.80 H: 3.90 N: 18.15.

Data for 1-[5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl]-3-(methylsulfonyl)imidazolidin-2-one (**3f**). Pale yellow, yield 40%, mp

Table 2. Physicochemical properties of synthesized compounds

Compound	SMILES	PSA	logD(7.4)
1	CS(=O)(=O)N1CCN(C(=O)NN)C1 = O	112.81	−2.25
2a	CS(=O)(=O)N1CCN(C(=O)NNC(=O)C2 = CC = C(F)C = C2)C1 = O	115.89	−1.13
2b	CS(=O)(=O)N1CCN(C(=O)NNC(=O)C2 = CC = C(Cl)C = C2)C1 = O	115.89	−0.66
2c	CS(=O)(=O)N1CCN(C(=O)NNC(=O)C2 = CC = C(C = C2)N(=O) = O)C1 = O	161.71	−1.45
2d	[H]C([H])C1 = CC = C(C = C1)C(=O)NNC(=O)N1CCN(C1 = O)S(C)(=O) = O	115.89	−0.57
2e	[H]C1 = CC = C(C = C1)C(=O)NNC(=O)N1CCN(C1 = O)S(C)(=O) = O	115.89	−1.13
2f	COC1 = CC = C(C = C1)C(=O)NNC(=O)N1CCN(C1 = O)S(C)(=O) = O	125.12	−1.34
2 g	CS(=O)(=O)N1CCN(C(=O)NNC(=O)C2 = CC = C(Br)C = C2)C1 = O	115.89	−0.5
2 h	CS(=O)(=O)N1CCN(C(=O)NNC(=O)C2 = C(Cl)C = C(Cl)C = C2)C1 = O	115.89	−0.19
3a	CS(=O)(=O)N1CCN(C1 = O)C1 = NN = C(O1)C1 = CC = C(F)C = C1	96.61	0.11
3b	CS(=O)(=O)N1CCN(C1 = O)C1 = NN = C(O1)C1 = CC = C(Cl)C = C1	96.61	0.57
3c	CS(=O)(=O)N1CCN(C1 = O)C1 = NN = C(O1)C1 = CC = C(C = C1)N(=O) = O	142.43	−0.09
3d	CC1 = CC = C(C = C1)C1 = NN = C(O1)N1CCN(C1 = O)S(C)(=O) = O	96.61	0.48
3e	[H]C1 = CC = C(C = C1)C1 = NN = C(O1)N1CCN(C1 = O)S(C)(=O) = O	96.61	−0.03
3f	COC1 = CC = C(C = C1)C1 = NN = C(O1)N1CCN(C1 = O)S(C)(=O) = O	105.84	−0.19
3 g	CS(=O)(=O)N1CCN(C1 = O)C1 = NN = C(O1)C1 = CC = C(Br)C = C1	96.61	0.74
3 h	CS(=O)(=O)N1CCN(C1 = O)C1 = NN = C(O1)C1 = C(Cl)C = C(Cl)C = C1	96.61	1.18
FCCP	[H]N(N = C(C#N)C#N)C1 = C([H])C([H]) = C(OC(F)(F)F)C([H]) = C1[H]	81.06	3.06

PSA, polar surface area.

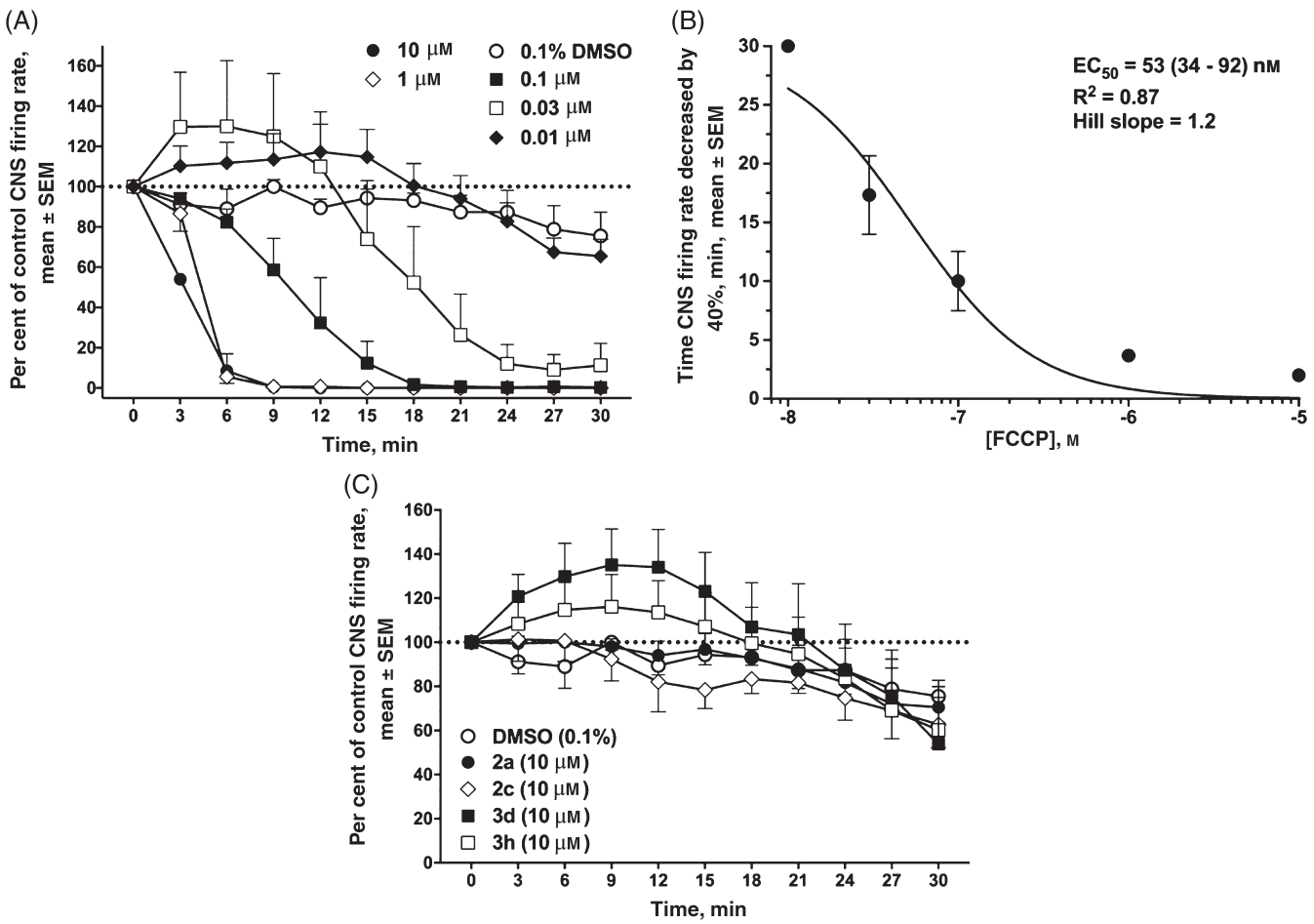


Figure 2. Effects of the mitochondrial uncoupler FCCP (A, B), with comparison to experimental compounds (C). The curve fit in B was constrained with the bottom = 0 and the top = 30.

265-266°C. IR υ_{max} (cm^{-1}): 3026, 2929, 1683, 1548, 1469, 1288, 831. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 3.36(s, 3H, -SO_2CH_3), 3.60 (s, 3H, OCH_3), 3.74-3.90 (m, 4H, -CH_2-), 7.10-7.90 (m, 4H, Ar-H). For $C_{13}H_{14}N_4O_5S$ (M.W.: 338.34 g/mol^{-1}), calcd: C: 46.15 H: 4.17 N: 16.56, found: 46.12 H: 4.18 N: 16.51.

Data for 1-[5-(4-bromophenyl)-1,3,4-oxadiazol-2-yl]-3-(methylsulfonyl)imidazolidin-2-one (**3 g**). White solid, yield 45%, mp 292-293°C. IR υ_{max} (cm^{-1}): 3020, 2928, 1683, 1550, 1473, 1280, 895. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 3.36 (s, 3H, -SO_2CH_3), 3.73-4.07 (m, 4H, -CH_2-), 7.70-8.07 (m, 4H, Ar-H). For $C_{12}H_{11}BrN_4O_4S$ (M.W.: 387.21 g/mol^{-1}), calcd: C: 37.22 H: 2.86 N: 14.47, found: C: 37.20 H: 2.82 N: 14.50.

Data for 1-[5-(2,4-dichlorophenyl)-1,3,4-oxadiazol-2-yl]-3-(methylsulfonyl)imidazolidin-2-one (**3 h**). White solid, yield 40%, mp 277-279°C. IR υ_{max} (cm^{-1}): 3026, 2933, 1683, 1585, 1467, 1259, 854. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 3.37 (s, 3H, -SO_2CH_3), 3.75-4.13 (m, 4H, -CH_2-), 7.57-7.80 (m, 3H, Ar-H). For $C_{12}H_{10}Cl_2N_4O_4S$ (M.W.: 377.20 g/mol^{-1}), calcd: C: 38.21 H: 2.67 N:14.85, found: C: 38.20 H: 2.64 N:14.90.

2.5 Mosquitoes and bioassay

Mosquitoes used for the adult topical bioassay were *Ae. aegypti* females of the Orlando strain (ORL) at 3 – 7 days post-emergence. ORL is susceptible to pesticides and has been maintained in a laboratory colony without additional field supplementation since 1952. The median lethal dose (LD_{50}) of permethrin for this strain is 0.1 – 0.2 ng. Standard rearing practices have been described previously and were used to produce females of 2.3 ± 0.3 mg each.[24]

The larval bioassay was performed in 96-well microtiter plates with first-instar larvae in a final volume of 200 µL per well as described previously.[24] Each larva was provided with 10 µL of the supernatant from a 2% solution of larval diet (1:1 alfalfa powder:pig chow). Two microliters of each compound diluted to 100 µg μL^{-1} in DMSO was added to the well and mixed gently with the larva. Further dilutions were made by the addition of 1, 0.5 or 0.2 µL of stock into other wells. For each assay, a positive control of permethrin and a negative control of DMSO were included. Assays were repeated at least three times on separate days.

Adult topical bioassays were performed as described previously.[24] A 0.5-µL aliquot of a 10 µg μL^{-1} initial screening dose in acetone was applied to cold anesthetized mosquitoes. Specimens were allowed to recover at 25 °C with access to 10% sucrose. Mortality was scored 24 h after application. Mosquitoes were counted as dead if they were without any movement or moribund, characterized by an inability to fly and stand upright. Three replicate bioassays were conducted on different days. Permethrin controls were included in all assays in addition to acetone, solvent-only controls.

Compounds that produced >80% mortality in pilot studies were subjected to further testing to develop LD_{50} values and 95% confidence intervals (CIs). A series of doses was applied to ORL *Ae. aegypti* females on three different days. Twenty-four-hour mortality for each dose was combined with results of dosing from other days for analysis. LD_{50} values and 95% CIs were determined by plotting dose – mortality data in a four-parameter logistic sigmoidal nonlinear regression as implemented in SigmaPlot v13 (SyStat Software, San Jose, CA, USA).

2.6 Electrophysiology

To assess the effects of experimental insecticides on nervous system function, third-instar larvae of *Drosophila melanogaster* were harvested from a colony of the wild-type Oregon R strain, kept at the University of Florida. Electrophysiological recordings from the larval central nervous system (CNS) were performed essentially as previously described.[25] The CNS was dissected in physiological saline containing (M) NaCl (0.157), KCl (0.003), $CaCl_2$ (0.002) and HEPES (0.004), at pH 7.2, and transected posterior to the cerebral lobes to eliminate the blood–brain barrier and facilitate penetration of chemicals into the CNS. A recording glass pipette suction electrode was used to monitor descending spike activity from several peripheral nerve trunks. Electrical signals were processed and converted to a rate using the PowerLab analog to digital converter hardware and LabChart 7 software (ADInstruments, Colorado Springs, CO, USA). Experimental compounds were then added to the saline bath in 1 µL of DMSO solution to a 1-ml bath and mixed by gentle pipetting. Drug effects over a 30-min period were assessed by taking 3-min average spike rates and plotting them against time.

2.7 Cellular respiration experiments

A Seahorse XFe96 Extracellular Flux Analyzer (Agilent Technologies, Santa Clara, CA, USA) was used to assess mitochondrial respiration and glycolytic activity in N2A cells according to previously published methods with minor modifications.[26] Briefly, cells were seeded into non-coated (N2A) XF^{e}96-well plates in Dulbecco's Modified Eagle Medium (DMEM) culture medium supplemented with 5% fetal bovine serum (FBS) and antibiotics approximately 24 h prior to the beginning of all experiments. On the day of the experiment, cells were treated with DMSO vehicle or compounds (30 µM) for 3 h, followed by metabolic flux analysis. Metabolic flux was assessed by quantification of the oxygen consumption rate (OCR), a measurement of mitochondrial respiration, or extracellular acidification rate (ECAR), a measurement of glycolytic function, in N2A cells.

One day prior to OCR and ECAR measurement assays, sensor cartridges were hydrated in XF calibrant (Agilent Technologies) and maintained in the XF PrepStation (Agilent Technologies) at 37 °C overnight. For the MitoStress test (Agilent Technologies) used to measure OCR the next day, cells were washed three times and incubated with bicarbonate free low-buffered assay medium for 1 h at 37 °C in a non-CO_2 incubator prior to beginning the assay. Oligomycin (1.0 µM), FCCP (0.6 µM), and rotenone/antimycinA (1:1, 1.3 µM) were added sequentially to cells over the course of the assay (final concentrations listed). For the glycolysis stress measured by ECAR, cells were washed three times with respiration buffer for 1 h at 37 °C in a CO_2 incubator prior to the assay. All data were collected and analyzed using Wave 2.3 software (Agilent Technologies).

2.8 Cheminformatics

The cheminformatics analysis of the pesticides was performed using MarvinSketch software (version 17.21.0; calculation module developed by ChemAxon; http://www.chemaxon.com/products/marvin/marvinsketch/, 2017) with an academic license.

3 RESULTS AND DISCUSSION

3.1 Synthesis

The synthetic route to the target compounds is outlined in Scheme 2. The structures of the compounds (**1, 2a – h, 3a – h**) were confirmed by IR, ^{1}H-NMR and elemental analysis. IR spectra of the compounds (**1, 2a – h**) showed N-H stretching (3221 – 3566)

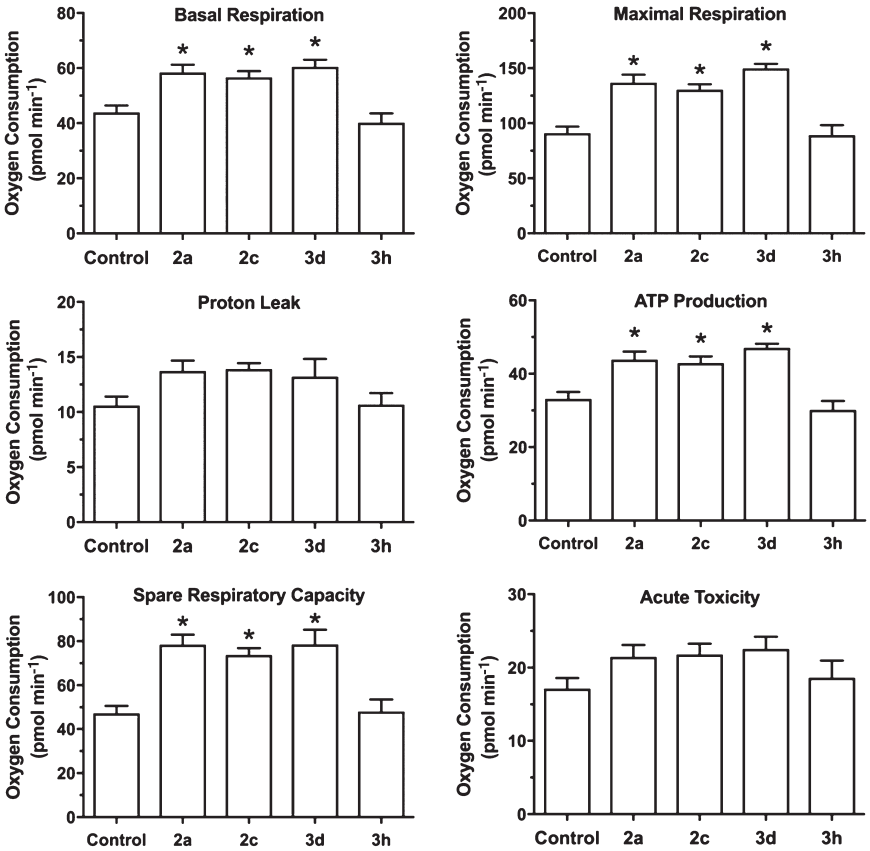


Figure 3. Results from the mitochondrial assays. N2A murine cells were treated with compounds (30 μM) for 3 h and then mitochondrial function was assayed. Each bar represents the mean ± standard deviation, where $n = 6 - 8$. *$P < 0.05$ signifies statistical significance.

bands. IR spectra of all compounds (**1, 2a – h, 3a – h**) showed C-H stretching (3009 – 3080), C = O stretching (1683 – 1697), aromatic ring C = C stretching (1467 – 1593) and S = O stretching (1230 – 1288) bands. The NH protons of hydrazide groups resonated as two different singlet peaks at 4.85 and 9.36 ppm. NH protons of carbohydrazide groups appeared as a singlet at 9.36 – 9.57 ppm. The aromatic protons displayed a multiplet at 7.28 – 8.84 ppm. The elemental analysis of compounds was in agreement with the proposed structures of the compounds.

3.2 Mosquito toxicity

Compounds were evaluated for larvicidal activity against *Ae. aegypti* and this initial screening activity indicated that none of the compounds produced mortality at concentrations of 1, 0.5, 0.25 and 0.1 μg μL^{-1} against first-instar *Ae. aegypti* larvae. The larval mortality from the solvent-only control was generally zero, whilst permethrin, the positive control, caused 100% larval mortality at 0.04 ng μL^{-1}. Table 1 shows adult topical testing with the ORL strain of *Ae. aegypti*, which indicated a broader range of toxicity which varied based on structure. For example, compounds **2a** having fluoro, **3d** carrying methyl, and **3f** containing a methoxy substituent on the aromatic ring were the least effective during initial screening, each causing approximately 50% or less mortality. In contrast, all of the other compounds produced >60% mortality at the 5 μg per mosquito screening dose. Compound **2b** was the most effective, killing nearly all the mosquitoes in three replicates. Slightly less effective were **3c**, **3 g** and **3 h,** followed by **2 g**, **3b**, **2c**, **2d, 3e**, **2e**, **2 h**, **3a**, and **2 f**.

All compounds with ≥80% mortality during an initial screening at 5 μg per mosquito were tested further over a range of concentrations to accurately define LD_{50} values. LD_{50} values, 95% CIs, and fitting parameters are shown in Table 1. All tested compounds had LD_{50} values between 2.3 and 5.0 μg per mosquito, with **3 h** and **2c** being the most effective. Although no significant correlation was observed between calculated logP (Clog P) values and activity, compound **3 h** having a chloro substituent and **2c** carrying a nitro group provided lipophilicity and these two compounds could possess increased cuticle/cell permeability. Compound **2c**, bearing a nitro group on the aromatic ring, and **3 h**, with double halogen groups at the 2,4 position on the phenyl ring, were the most promising compounds.

N-acylated hydrazines and the 1,3,4-oxadiazole ring showed similar activity. Both *N*-acylated hydrazines (**2b**, **2c**, **2d**, **2 g**) and their corresponding oxadiazole derivatives (**3b**, **3c**, **3 g**, **3 h**) were effective. Activity was processed by ring cyclization, with hydrogen donor and acceptor groups protected during synthesis.

3.3 Mode of action studies

Most of the major pesticides have been shown to cross-react with mitochondria. Considering the importance of such cross-reactions for both selectivity and a possible targeted approach, we evaluated the compound pairs from series **2** and **3** in preliminary mode of action experiments focused on mitochondrial activity, while comparing with the mitochondrial uncoupling agent, FCCP, as standard. The experimental compound **2a** showed low toxicity (Table 1) and **2c** showed high toxicity (Table 1). The compounds have some minor structural similarity to FCCP (Scheme 2

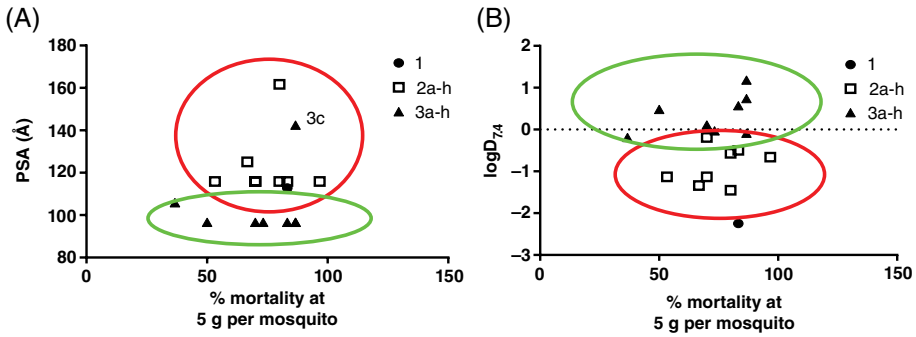


Figure 4. Correlation between the insecticidal activities of the two compound series and the physicochemical properties polar surface area (PSA) and logD7.4. As can be seen, there are two main clusterings of the compounds' chemical structural properties, both of which are related to the ability of compounds to reach the target site inside insects.

and Table 2), both having adjacent nitrogen atoms, with the one nearest the phenyl ring having calculated pKa values of 6.7 and 6.4, respectively, similar to the value of 6.4 derived for FCCP (Scheme 2).[27] In Figure 4, the compounds were correlated with the polar surface area (PSA) which is defined as the sum of surfaces of polar atoms. The two compound groups show clear grouping, except for **3c** which seems to have a propensity to show a higher PSA as compared with the rest of the compounds in its group. Similarly, the compounds grouped naturally when considering logD at pH 7.4, where logD = logP +Pka at pH 7.4. Both these properties relate to the ability of the compounds to reach their target sites in the insect or in the off-target mammalian cell. Also evaluated were the closed ring analogs **3d** and **3 h**, having similar low- and high-toxicity profiles, but no acidic protons bonded to nitrogen. Electrophysiological experiments on the fly CNS found that FCCP caused a small and variable increase in nerve firing at 10 – 30 nM, with the dominant effect a reduction in nerve firing rate (Figure 2A), consistent with a disruption in ATP generation. Nonlinear curve fit of concentration – response data gave an IC_{50} value (concentration needed to inhibit 50% of control mosquitoes) in the mid nanomolar range (Figure 2B). The experimental compounds **3d** and **3 h** displayed an increase in nerve firing followed by a return to control levels. The other two compounds, **2a** and **2c**, had little effect. Thus, effects on nerve firing in *D. melanogaster* third-instar larvae did not correlate with mosquito toxicity.

The effects of the compounds on mitochondrial respiration were tested in mammalian cells. As can be seen from Figure 3, three of the four compounds had effects on some mitochondrial respiration parameters (**2a**, **2c** and **3d**), while one was inactive (**3 h**). The active compounds increased basal respiration, maximal respiration stimulated by FCCP, respiration linked to ATP production, and spare respiratory capacity. There was no significant change in proton leak and acute toxicity. These results suggest an increase in cell stress reflected in mitochondrial physiology. Compound **3 h** did not have any effects on the mitochondrial activity of the cells, despite being one of the most toxic compounds to mosquitoes. Thus, while mitochondrial effects were observed, there was not a clear structure – toxicity relationship that could be derived from these experiments.

4 CONCLUSION

In this pilot study, a series of new oxadiazole derivatives containing a 1,3,4-oxadiazole group bearing an imidazolidine moiety were synthesized and the structures were characterized by ^{1}H-NMR, IR, and elemental analysis. The structure–activity relationship of these 17 compounds indicated that, of the phenyl substituents tested, chloro, nitro, methyl, and bromo substituents on **2b**, **2c**, **2d**, **2 g**, **3b**, **3c**, **3 g** and **3 h** were effective in increasing adulticidal activity, and further structural variation may lead to the development of new effective insecticides. A limited series of mode of action studies failed to explain the lethality in mosquitoes by neurotoxic or mitochondrial respiratory mechanisms.

Disclosure statement

No potential conflict of interest is reported by the authors.

ACKNOWLEDGEMENTS

This study was partly funded by the Deployed War-Fighter Protection Research Program via grants from the US Department of Defense through the Armed Forces Pest Management Board to J. J. Becnel (60-0208-6-001) and J. R. Bloomquist (58-0208-0-068 and 58-0208-5-001), as well as grants from the National Institute of General Medical Sciences (U54GM104942 and P20 GM109098) to W. J. Geldenhuys. We thank Miss Jessica Louton (USDA-ARS, CMAVE, Gainesville, FL, USA) for mosquito bioassays.

REFERENCES

1 Nauen R, Insecticide resistance in disease vectors of public health importance. *Pest Manag Sci* **63**:628–633 (2007).
2 Benelli G, Canale A, Higuchi A, Murugan K, Pavela R and Nicoletti M, The recent outbreaks of Zika virus: Mosquito control faces a further challenge. *Asian Pac J Trop Dis* **6**:253–258 (2016).
3 Melo MC, Donalisio MR and Freitas AR, Zika virus, from discovery to the present days: Bibliometric review study. *International Journal of Current Research* **9**:46728–46740 (2017).
4 Gross AD, Tabanca N, Islam R, Ali A, Khan IA, Kaplancikli ZA *et al.*, Toxicity and synergistic activities of chalcones against Aedes aegypti and Drosophila melanogaster. *J Med Entomol* **54**:382–386 (2016).
5 Swale DR, Engers DW, Bollinger SR, Gross A, Inocente EA, Days E *et al.*, An insecticide resistance breaking mosquitocide targeting inward rectifier potassium channels in vectors of Zika virus and malaria. *Nature* **6**:1–11 (2016).
6 Vale N, Collins MS, Gut J, Ferraz R, Rosenthal PJ, Cushion MT *et al.*, Anti-*Pneumocystis carinii* and antiplasmodial activities of primaquine-derived imidazolidin-4-ones. *Bioorg Med Chem Lett* **18**:485–488 (2008).
7 Fernandes I, Vale N, Freitas V, Moreira R, Mateus N and Gomes P, Anti-tumoral activity of imidazoquines, a new class of antimalarials derived from primaquine. *Bioorg Med Chem Lett* **19**:6914–6917 (2009).
8 Zhuang AX, Zhang YX, Zhang H and Liu ZW, The insecticidal activity and action mode of an imidacloprid analogue, 1-(3-pyridylmethyl)-2-nitroimino-imidazolidine. *Insect Sci* **23**:688–694 (2016).

9 Nishimura K, Tanaka M, Iwaya K and Kagabu S, Relationship between insecticidal and nerve-excitatory activities of imidacloprid and its alkylated congeners at the imidazolidine NH site. *Pestic Biochem Phys* **62**:172–178 (1998).

10 Liu Q, Zhu R, Gao S, Ma SH, Tang HJ, Yang JJ *et al.*, Structure-based bioisosterism design, synthesis, insecticidal activity and structure-activity relationship (SAR) of anthranilic diamide analogues containing 1,2,4-oxadiazole rings. *Pest Manag Sci* **73**:917–924 (2017).

11 Zhou L, Wang PY, Zhou J, Shao WB, Fang HS, Wu ZB *et al.*, Antimicrobial activities of pyridinium-tailored pyrazoles bearing 1,3,4-oxadiazole scaffolds. *Journal of Saudi Chemical Society* **17**:1–61 (2017).

12 Zhang Y, Liu XH, Zhan YZ, Zhang LY, Li ZM, Li YH *et al.*, Synthesis and biological activities of novel 5-substituted-1,3,4-oxadiazole Mannich bases and bis-Mannich bases as ketol-acid reductoisomerase inhibitors. *Bioorg Med Chem Lett* **26**:4661–4665 (2016).

13 Dai H, Chen J, Li G, Ge S, Shi Y, Fang Y *et al.*, Design, synthesis and bioactivities of novel oxadiazole-substituted pyrazoleoximes. *Bioorg Med Chem Lett* **27**:950–953 (2017).

14 Wu W, Chen Q, Tai A, Jiang G and Ouyang G, Synthesis and antiviral activity of 2-substituted methylthio-5-(4-amino-2-methylpyrimidine-5-yl)-1,3,4-oxadiazole derivatives. . *Bioorg Med Chem Lett* **25**:2243–2246 (2015).

15 Chaves JD, Tunes LG, Franco CH, Francisco TM, Correa CC, Murta SMF *et al.*, Novel gold complexes with 5-phenyl-1,3,4-oxadiazole-2-thione and phosphine as potential anticancer and antileishmanial agents. *Eur J Med Chem* **127**:727–739 (2017).

16 Rathore A, Sudhakar R, Ahsan MJ, Ali A, Subbarao N, Jadav SS *et al.*, *In vivo* anti-inflammatory activity and docking study of newly synthesized benzimidazole derivatives bearing oxadiazole and morpholine rings. *Bioorg Chem* **70**:107–117 (2017).

17 Bajaj S, Asati V, Singh J and Roy PP, 1,3,4-oxadiazoles: An emerging scaffold to target growth factors, enzymes and kinases as anticancer agents. *Eur J Med Chem* **97**:124–141 (2015).

18 Tabanca N, Ali A, Bernier UR, Khan IA, Koçyiğit-Kaymakçıoğlu B, Oruç-Emre EE *et al.*, Biting Deterrence and Insecticidal activity of hydrazide–hydrazones and their corresponding 3-acetyl-2,5-disubstituted-2,3-dihydro-1,3,4-oxadiazoles against *Aedes aegypti*. *Pest Manag Sci* **69**:703–708 (2013).

19 Benelli G, Canale A, Higuchi A, Murugan K, Pavela R and Nicoletti M, The recent outbreaks of Zika virus: Mosquito control faces a further challenge. *Asian Pac J Trop Dis* **6**:253–258 (2016).

20 Masi M, Cala A, Tabanca N, Cimmino A, Green IR, Bloomquist JR *et al.*, Alkaloids with Activity against the Zika Virus Vector *Aedes aegypti* (L.) - Crinsarnine and Sarniensinol, Two New Crinine and Mesembrine Type Alkaloids Isolated from the South African *Nerine sarniensis*. *Molecules* **21**: 1432 (2016).

21 Jha KK, Samad A, Kumar Y, Shaharyar M, Khosa RL, Jain J *et al.*, Design, synthesis and biological evaluation of 1,3,4-oxadiazole derivatives. *Eur J Med Chem* **45**:4963–4967 (2010).

22 Zhou ZZ, Ge BC, Chen YF, Shi XD, Yang XM and Xu JP, Catecholic amides as potential selective phosphodiesterase 4D inhibitors: Design, synthesis and pharmacological evaluation and structure-activity relationships. *Bioorg Med Chem* **23**:7332–7339 (2015).

23 Hamciuc C, Hamciuc E, Homocianu M, Nicolescu A and Carja ID, Blue light-emitting polyamide and poly (amide-imide)s containing 1,3,4-oxadiazole ring in the side chain. *Dyes Pigments* **114**:110–123 (2015).

24 Estep AS, Becnel JJ and Lee ST, Toxicity of compounds isolated from white snakeroot (*Ageratina altissima*) to adult and larval yellow fever mosquitoes (*Aedes aegypti*). *Nat Prod Commun* **11**:1675–1677 (2016).

25 Jiang S, Tsikolia M, Bernier U and Bloomquist JR, Mosquitocidal activity and mode of action of the isoxazoline fluralaner. *Int J Environ Res Public Health* **14**:154 (2017).

26 Logan SI, Geldenhuys WJ, Enrick MK, Stevanov KM, Caroll RT, Ohanyan VA *et al.*, Novel thiazolidinedione mitoNEET ligand-1 acutely improves cardiac stem cell survival under oxidative stress. *Basic Res Cardiol* **110**:19 (2015).

27 Anonymous, ACE and JChem acidity and basicity calculator. [Online]. Available: https://ace.chem.illinois.edu/ace/public/pKa.jsp (2017).

