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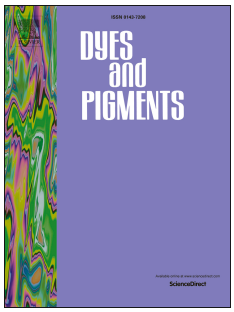

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Evaluation of fluorogenic aminonaphthalenesulfonamides and 6-hydrazinobenz[*de*]isoquinoline-1,3-diones for the detection of bacteria

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Abstract

New fluorogenic enzyme substrates were synthesized by the coupling of aminonaphthalenesulfonamides or 6-hydrazinobenz[*de*]isoquinoline-1,3-diones with β-alanine. The 6-hydrazinobenz[*de*]isoquinoline-1,3-diones were also condensed with a range of aryl aldehydes to give the corresponding hydrazones. The photophysical properties of the synthesized amines and hydrazines and their amide, hydrazide and hydrazone derivatives, were examined and they were also incorporated into Columbia agar in order to determine their potential for the detection of pathogenic bacteria.

Keywords

Aminonaphthalenesulfonamides, 6-hydrazinobenz[*de*]isoquinoline-1,3-diones, hydrazones, fluorescence, pathogenic bacteria.

1. Introduction

Antimicrobial resistance has been a major obstacle to the effective treatment of bacterial infections for several decades and has recently become a serious threat to global public health, contributing to over 720,000 potentially preventable healthcare-associated infections (HAIs) in the US *p.a.*[1] and more than 177,000 *p.a.* in Australia.[2] Although a natural phenomenon, antimicrobial resistance has been accelerated by the misuse of antimicrobial agents, allied to a lack of new agents to replace those which have become ineffective (only

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two truly novel antibacterial agents have been approved in the last 30 years). Antibiotic stewardship programs have been introduced worldwide in attempts to improve patient outcomes, reduce adverse sequelae associated with antimicrobial use, and to preserve the ever more limited array of antibacterials which infectious disease clinicians have at their disposal.[3, 4] In addition, effective prevention and control of infection can help to reduce the spread of antibiotic resistance, and this can be informed by universal screening protocols,[5] which are ideally based upon rapid, simple, and cost-effective techniques for the detection and identification of pathogenic bacteria.

One of the most commonly employed techniques for the detection and identification of bacteria involves the use of chromogenic culture media,[6] which employ an initially non-coloured substrate, consisting of an enzyme-targeting moiety covalently attached to a chromogen. In the presence of an enzyme which is specific to a particular bacterium, the covalent bond is cleaved, releasing the chromogen, Figure 1, and thus allowing the detection and identification of this bacterium.

For example, chromID™ *P. aeruginosa*, exploits β-alanyl-1-pentyl resorufamine **1**, which is cleaved by β-alanyl aminopeptidase to give the chromogen 1-pentylresorufamine **2**, Figure 1, to enable the rapid identification of *P. aeruginosa* in patients with cystic fibrosis before conversion into a difficult-to-treat mucoid phenotype.[7] Other chromogenic media have been employed for the detection of multi-resistant organisms, such as methicillin-resistant *Staphylococcus aureus* (*e.g.* CHROMagar™ MRSA),[9] vancomycin-rsistant enterococci (*e.g.* VRE-BMX),[10] and NDM-1 (carbapenemase) producing Enterobacteriaceae.[11]

Although sufficiently specific to allow presumptive species identification, a limiting factor in the use of chromogenic media is the time taken (typically 24-48 hours) for the development of sufficient quantities of the indicative colour of the chromogen, which is essential for reliable detection against the usually lightly coloured background. This limitation can be addressed through the development of fluorogenic media, since the detection of fluorescence is inherently more sensitive than the perception of colour change — media containing fluorogenic substrates should lead to a reduction in the time required for bacterial detection.[12]

In order to develop fluorogenic media for the detection of bacteria, the reliable detection of the enzymatically-released fluorophores is necessary, and this can be facilitated by two methods; (i) either an off-to-on mechanism analogous to chromogenic media or (ii) as the

result of a significant change in the observed fluorescent emission wavelengths between the substrate and subsequent fluorogen . In addition to displaying intense fluorescence, the ideal fluorogen should be non-growth inhibitory and retained within bacterial cells / colonies, while the substrate should be water soluble and readily taken up by bacterial cells.

Based on their reported fluorescent properties, we identified aminonaphthalenesulfonamide (ANS) and 6-hydrazinobenz[*de*]isoquinoline-1,3-dione (6-HBI) cores as potential fluorogens for the detection of bacterial enzymes, Figure 2. Aminonaphthalenesulfonamides (ANS) have previously been reported as useful fluorogenic substrates for proteolytic enzymes involved in fibrinolysis and blood coagulation, and the quantitation of Lys-plasmin.[13] Similiarly, benz[*de*]isoquinoline-1,3-diones have been used as enzymatic markers for dipeptidyl peptidase IV and tripeptidyl peptidase I activity in rodents,[14] as a probe for nitroreductase activity for the detection of hypoxic cells,[15, 16] and as a marker for the detection of *Mycobacterium tuberculosis*.[17]

In order to evaluate their potential for the detection of bacteria in clinical samples, various derivatives of both sets of fluorescent cores were designed, synthesised and coupled to a suitable amino acid. β-Alanine was initially chosen as the amino acid moiety in order to demonstrate the utility of these fluorogens in bacterial systems by targeting the β-alanyl aminopeptidase activity of *P. aeruginosa*. The hydrolysis of the amide bond in the presence of this bacterium would result in the release of the fluorogen. In the case of the 6-HBI core, a secondary reaction, with a range of *para*-substituted benzaldehydes and cinnamaldehyde, was also envisaged in order to enhance the fluorescence observed as a result of the extension of the conjugation. Both fluorescent core molecules would be modified through the introduction of substituents with different alkyl (R) chain lengths in order to ascertain the optimum uptake, adherence, and lack of growth inhibition.

Synthesis of the desired fluorophores was first achieved using the methods described below and their subsequent coupling to Boc-β-alanine was achieved with the same symmetrical anhydride method for both core molecules.

2. Experimental

2.1 General

All reagents and solvents were purchased from Sigma-Aldrich, Alfa Aesar, and ChemSupply, and used without any further purification or treatment. Thin layer chromatography was performed on Grace Reveleris® Silica Aluminum-backed TLC Plates (UV254). ^{1}H and ^{13}C

NMR spectra were acquired on a Varian 400MR at 400 MHz and 100 MHz, respectively. Coupling constants (*J*) are in Hertz (Hz), chemical shifts (δ) are expressed in parts per million (ppm) and reported relative to residual solvent peaks. Melting points were obtained on a Stuart Scientific SMP 10. Low resolution mass spectra were obtained on TSQ Quantum Access Max (Triple Quadrupole) LCMS/MS in positive ion mode. High resolution mass spectra were obtained on a Bruker 7T Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (FTICR) in positive ion mode. Infrared spectra were recorded on a Shimadzu FTIR-8400S and Shimadzu IRTracer-100. Elemental analyses were obtained from the Campbell Microanalytical Laboratory in the University of Otago, NZ.

2.2 General procedure for the preparation of acetamides 6a-e

Imide **4** was prepared by the method of Xu *et al.*[18] and sulfonyl chloride **5** by the method of Guan *et al*..[19]

To a solution of **5** (1 equiv.) in DCM, triethylamine (2 equiv.) and the appropriate amine (1.5 equiv.) were added. The resulting solution was then left to stir at room temperature overnight. Upon completion, the organic layer was washed with 1M HCl (3 × 20 mL), water (3 × 20 mL) and brine (3 × 20 mL), then dried over Na_2SO_4. The solvent was removed *in vacuo* and the crude product was purified by column chromatography, eluting with ethyl acetate: hexane (25:75 to 75:25) to give acetamides **6a-e**.

2.3 General procedure for the deacetylation of acetamides 6a-e to produce sulfonamides 7a-e

The appropriate acetamide **6a-e** was dissolved in methanol / 5M aqueous NaOH mixture (3:2 v/v) and kept at 85 °C. Upon completion of the reaction, the methanol was removed *in vacuo* and the aqueous residue was extracted with DCM (6 × 25mL). After drying over Na_2SO_4, the organic solvent was removed and the crude product was purified by column chromatography on silica, eluting with ethyl acetate: hexane (50:50 to 60:40) to yield the desired sulfonamides **7a-e**.

2.4 General method for preparation of 6-bromo-1*H*-benz[*de*]isoquinoline-1,3(2*H*)-diones 9

4-Bromo-1,8-naphthalic anhydride **8** (1 equiv.) and the corresponding amine (1 equiv.) were refluxed in ethanol (25 mL) for 2 hours. The solution was then allowed to cool to room temperature and the precipitate which formed was collected by filtration to give the desired product **9a-c**.

2.5 General method for the preparation of 6-hydrazino-1*H*-benz[*de*]isoquinoline-1,3(2*H*)-diones 10

The 2-alkyl-6-bromobenz[*de*]isoquinolin-1,3(2*H*)-dione **9a-c** (1 equiv.) was reacted with hydrazine hydrate (2 equiv.) in 2-butanol (25 mL) under reflux. After 2 hours, additional hydrazine hydrate (2 equiv.) was added to the solution and after an additional 4 hours, the solution was allowed to cool to room temperature. The precipitate which formed was washed with diethyl ether to give the desired product **10a-c**.

2.6 General procedure for the coupling with Boc-β-alanine to give sulfonamides 11a-e and hydrazides 13a-c

Boc-β-alanine (8 equiv.) was dissolved in DCM and cooled to 0 °C. To this solution, DCCI (4 equiv.) was added and the mixture was allowed to warm to room temperature and left to stir for 1 hour. This mixture was then filtered into a flask containing the appropriate amine **7** or hydrazine **10** (1 equiv.), EDIPA (1.2 equiv.) and DMAP (0.6 equiv.) in DCM. The resulting solution was then heated to 50 °C and left to stir. Upon completion of the reaction, the organic layer was washed with saturated $NaHCO_3$ (3 × 20 mL), 1M aqueous HCl (3 × 20 mL) and water (3 × 20 mL) then dried over Na_2SO_4. The solvent was removed *in vacuo* and the crude products **11a-e** were purified by column chromatography on silica, eluting with ethyl acetate: hexane (20:80 to 50:50).

For the coupling to the hydrazinobenz[*de*]isoquinolin-1,3(2*H*)diones **10a-c**, after 3 hours, additional Boc-β-Alanine anhydride (4 equiv.) was added to the solution. After a further 20 hours, the solvent was removed *in vacuo* and the residue was purified *via* column chromatography on silica gel. Recrystallization from ethanol gave the Boc-protected substrates **13a-c**.

2.6.1 tert-*Butyl [3-oxo-3-{5-(*N-*propylsulfamoyl)naphthalen-1-yl}aminopropyl)]carbamate (11a)*

Yield of **11a**: 20%; White solid; mp 177-179 °C; FTIR (neat, *ν*/cm^{-1}): 3291 (NH), 3271 (NH), 1684 (C=O), 1653 (C=O), 1597 (C=C, aromatic), 1290 (S=O), 1153 (S=O); ^{1}H NMR (400 MHz, CD_3OD) δ: 0.76 (3H, t, *J* = 7.4 Hz, CH_3-3′), 1.36 (2H, sextet, *J* = 7.2 Hz, CH_2-2′), 1.46 (9H, s, $C(CH_3)_3$), 2.75 (2H, t, *J* = 6.7 Hz, CH_2-α), 2.79 (2H, t, *J* = 7.0 Hz, CH_2-1′), 3.48 (2H, t, *J* = 6.7 Hz, CH_2-β), 7.62-7.73 (3H, m), 8.24 (1H, d, *J* = 6.4 Hz), 8.31 (1H, d, *J* = 8.4 Hz), 8.65 (1H, d, *J* = 8.4 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ: 11.45 (CH_3, C-3′), 24.0 (CH_2, C-2′), 28.8 (3 × CH_3, $C(CH_3)_3$), 37.8 (CH_2, C-α), 38.1 (CH_2, C-β), 45.8 (CH_2, C-1′), 80.2 (quat.,

C(CH_3)$_3$), 124.6 (CH), 125.4 (CH), 125.5 (CH), 128.5 (CH), 129.5 (CH), 130.3 (quat.), 130.4 (CH), 131.3 (quat.), 135.1 (quat.), 137.5 (quat.), 158.5 (C=O, carbamate CO), 173.6 (C=O, amide CO); ESI-MS = 436 (M + H). *Anal. Calcd. for* $C_{21}H_{29}N_3O_5S$ (435): C, 57.91; H, 6.71; N, 9.65. Found: C, 57.99; H, 6.92; N, 9.72%.

2.6.2 tert-*Butyl [3-oxo-3-{(5-(*N-*butylsulfamoyl)naphthalen-1-yl}aminopropyl]carbamate (11b)*

Yield of **11b**: 51%; White solid; mp 175-178 °C; FTIR (neat, *v*/cm^{-1}): 3308 (NH), 3265 (NH), 1680 (C=O), 1651 (C=O), 1620 (C=C, aromatic), 1290 (S=O), 1150 (S=O); ^{1}H NMR (400 MHz, CD_3OD) δ: 0.74 (3H, t, *J* = 7.4 Hz, CH_3-4′), 1.18 (2H, sextet, *J* = 7.4 Hz, CH_2-3′), 1.32 (2H, quintet, *J* = 7.2 Hz, CH_2-2′), 1.46 (9H, s, C(CH_3)$_3$), 2.745 (2H, t, *J* = 6.7 Hz, CH_2-α), 2.82 (2H, t, *J* = 6.8 Hz, CH_2-1′), 3.48 (2H, t, *J* = 6.7 Hz, CH_2-β), 7.62-7.73 (3H, m), 8.24 (1H, d, *J* = 6.8 Hz), 8.31 (1H, d, *J* = 8.4 Hz), 8.64 (1H, d, *J* = 8.4 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ: 13.8 (CH_3, C-4′), 20.65 (CH_2, C-3′), 28.8 (3 × CH_3), 32.7 (CH_2, C-2′), 37.8 (CH_2, C-α), 38.05 (CH_2, C-β), 43.6 (CH_2, C-1′), 80.2 (quat., *C*(CH_3)$_3$), 124.55 (CH), 125.4 (CH), 125.5 (CH), 128.5 (CH), 129.5 (CH), 130.3 (quat.), 130.4 (CH), 131.3 (quat.), 135.1 (quat.), 137.4 (quat.), 158.5 (C=O, carbamate CO), 173.6 (C=O, amide CO); ESI-MS = 450 (M + H). *Anal. Calcd. for* $C_{22}H_{31}N_3O_5S$ (449): C, 58.78; H, 6.95; N, 9.35. Found: C, 58.80; H, 7.13; N, 9.40%.

2.6.3 tert-*Butyl [3-oxo-3-{(5-(*N-*pentylsulfamoyl)naphthalen-1-yl}aminopropyl]carbamate (11c)*

Yield of **11c**: 27%; White solid; mp 166-170 °C; FTIR (neat, *v*/cm^{-1}): 3320 (NH), 3269 (NH), 1686 (C=O), 1653 (C=O), 1622 (C=C, aromatic), 1287 (S=O), 1153 (S=O); ^{1}H NMR (400 MHz, CD_3OD) δ: 0.74 (3H, t, *J* = 6.8 Hz, CH_3), 1.09-1.14 (4H, m, 2 × CH_2, 2 × H-3′ plus 2 × H-4′), 1.29-1.36 (2H, m, CH_2-2′), 1.46 (9H, s, C(CH_3)$_3$), 2.75 (2H, t, *J* = 6.8 Hz, CH_2-α), 2.82 (2H, t, *J* = 7.0 Hz, CH_2-1′), 3.48 (2H, t, *J* = 6.6 Hz, CH_2-β), 7.62-7.73 (3H, m), 8.24 (1H, d, *J* = 7.2 Hz), 8.31 (1H, d, *J* = 8.4 Hz), 8.64 (1H, d, *J* = 8.4 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ: 14.2 (CH_3, C-5′), 23.1 (2 × CH_2, C-3′ and C-4′), 28.8 (3 × CH_3), 30.3 (CH_2, C-2′), 37.8 (CH_2, C-α), 38.1 (CH_2, C-β), 43.9 (CH_2, C-1′), 80.2 (quat., *C*(CH_3)$_3$), 124.6 (CH), 125.4 (CH), 125.5 (CH), 128.5 (CH), 129.5 (CH), 130.3 (quat.), 130.4 (CH), 131.3 (quat.), 135.2 (quat.), 137.5 (quat.), 158.5 (C=O, carbamate CO), 173.6 (C=O, amide CO); ESI-MS = 486 (M + Na). HRMS *Calcd. for* $C_{23}H_{33}N_3O_5SNa$: 486.2033. Found: 486.2038.

2.6.4 tert-*Butyl [3-oxo-3-{5-(*N-*octylsulfamoyl)naphthalen-1-yl}aminopropyl]carbamate (11d)*

Yield of **11d**: 37%; White solid; mp 175-177 °C; FTIR (neat, *ν*/cm^{-1}): 3275 (NH), 1676 (C=O), 1653 (C=O), 1595 (C=C, aromatic), 1321 (S=O), 1150 (S=O); ^{1}H NMR (400 MHz, CD_3OD) δ: 0.84 (3H, t, *J* = 7.2 Hz, CH_3), 1.07-1.15 (8H, m, 4 × CH_2), 1.20-1.32 (4H, m, 2 × CH_2), 1.44 (9H, s, $C(CH_3)_3$), 2.72 (2H, t, *J* = 6.7 Hz, CH_2-α), 2.80 (2H, t, *J* = 7.0 Hz, CH_2-1′), 3.46 (2H, t, *J* = 6.7 Hz, CH_2-β), 7.59-7.72 (3H, m), 8.22 (1H, d, *J* = 6.8 Hz), 8.29 (1H, d, *J* = 8.4 Hz), 8.61 (1H, d, *J* = 8.4 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ: 14.4 (CH_3, C-8′), 23.7 (CH_2), 27.55 (3 × CH_3), 28.8 (CH_2), 30.1 (CH_2), 30.2 (CH_2), 30.6 (CH_2), 32.9 (CH_2), 37.8 (CH_2, C-2″), 36.6 (CH_2, C-3″), 42.4 (CH_2, C-1′), 80.23 (quat., *C*$(CH_3)_3$), 124.5 (CH), 125.3 (CH), 125.5 (CH), 128.5 (CH), 129.5 (CH), 130.3 (quat.), 130.4 (CH), 131.2 (quat.), 135.15 (quat.), 137.5 (quat.), 158.5 (C=O, carbamate CO), 173.55 (C=O, amide CO); ESI-MS = 506 (M + H); *Anal. Calcd. for* $C_{26}H_{39}N_3O_5S$ (505): C, 61.76; H, 7.77; N, 8.31. Found: C, 61.76; H, 8.00; N, 8.34%.

2.6.5 tert-*Butyl [3-{2-(1,3-dioxo-2-propyl-2,3-dihydro-1*H-*benzo[*de*]isoquinolin-6-yl)hydrazinyl}-3-oxopropyl]carbamate (13a)*

Yield of **13a**: 21%; Light yellow solid; mp 193-195 °C; FTIR (neat, *ν*/cm^{-1}): 3336 (NH), 3325 (NH), 3279 (NH), 1684 (C=O), 1655 (amide I), 1647 (C=O), 1585 (amide II), 1528 (C=C); ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ: 0.97 (3H, t, *J* = 7.6 Hz, H-3′), 1.45 (9H, s, $C(CH_3)_3$), 1.72 (2H, sextet, *J* = 7.6 Hz, H-2′), 2.66 (2H, t, *J* = 6.8 Hz, CH_2-α), 3.44 (2H, q, *J* = 6.8 Hz, CH_2-β), 4.04-4.08 (2H, m, H-1′), 6.08 (1H, broad s, carbamate NH), 7.11 (1H, d, *J* = 8.4 Hz, H-5), 7.60 (1H, dd, *J* = 8.4, 7.6 Hz, H-8), 8.34 (1H, d, *J* = 8.4 Hz, H-4), 8.43 (1H, dd, *J* = 7.6, 1.2 Hz, H-9), 8.54 (1H, dd, *J* = 8.4, 1.2 Hz, H-7), 9.16 (1H, broad s, amide NH); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ: 12.8 (CH_3, C-3′), 23.1 (CH_2, C-2′), 29.7 (3 × CH_3, C(*C*$H_3)_3$), 35.9 (CH_2, CH_2-α), 38.7 (CH_2, CH_2-β), 43.0 (CH_2, C-1′), 79.8 (quat., *C*$(CH_3)_3$), 107.6 (CH, C-5), 114.6 (quat., C-3a), 121.5 (quat., C-6a), 124.7 (quat., C-9a), 127.0 (CH, C-8), 129.0 (CH, C-7), 131.1 (quat., C-3b), 132.3 (CH, C-9), 134.9 (CH, C-4), 152.1 (quat., C-6), 157.6 (C=O, carbamate CO), 165.1 (C=O, C-3), 165.6 (C=O, C-1), 172.9 (C=O, amide CO); ESI-MS = 442 (M + H).

2.6.6 tert-*Butyl [3-{2-(1,3-dioxo-2-pentyl-2,3-dihydro-1*H-*benzo[*de*]isoquinolin-6-yl)hydrazinyl}-3-oxopropyl]carbamate (13b)*

Yield of **13b**: 19%; Yellow-brown solid; mp 150-154 °C; FTIR (neat, *v*/cm^{-1}): 3357 (NH), 3312 (NH), 3221 (NH), 1688 (C=O), 1674 (amide I), 1639 (C=O), 1614 (amide II), 1587 (C=C), 1519 (C=C); ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ: 0.93 (3H, t, *J* = 7.2 Hz, H-5′), 1.38-1.41 (4H, m, H-3′, H-4′), 1.46 (9H, s, $C(CH_3)_3$), 1.72 (2H, quintet, *J* = 7.6 Hz, H-2′), 2.70 (2H, t, *J* = 6.4 Hz, CH_2-α), 3.47 (2H, q, *J* = 6.4 Hz, CH_2-β), 4.07 (2H, t, *J* = 7.6 Hz, H-1′), 6.13 (1H, broad s, carbamate NH), 7.06 (1H, d, *J* = 8.0 Hz, H-5), 7.39 (1H, dd, *J* = 8.4, 7.6 Hz, H-8), 8.26-8.29 (2H, m, H-4, H-9), 8.38 (1H, dd, *J* = 8.4, 0.8 Hz, H-7), 9.08 (1H, broad s, NH), 9.49 (1H, broad s, NH); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ: 15.3 (CH_3, C-5′), 24.1 (CH_2, C-4′), 29.5 (CH_2, C-2′), 29.7 (3 × CH_3, $C(CH_3)_3$), 31.1 (CH_2, C-3′), 36.0 (CH_2, CH_2-α), 38.7 (CH_2, CH_2-β), 41.4 (CH_2, C-1′), 80.0 (quat., $C(CH_3)_3$), 107.3 (CH, C-5), 114.6 (quat., C-3a), 121.2 (quat., C-6a), 124.4 (quat., C-9a), 126.8 (CH, C-8), 128.8 (CH, C-7), 130.8 (quat., C-3b), 131.9 (CH, C-9), 134.8 (CH, C-4), 151.9 (quat., C-6), 157.7 (C=O, carbamate CO), 165.0 (C=O, C-3), 165.5 (C=O, C-1), 173.4 (C=O, amide CO); ESI-MS = 469 (M + H).

2.6.7 tert-*Butyl [3-{2-(1,3-dioxo-2-benzyl-2,3-dihydro-1*H*-benzo[*de*]isoquinolin-6-yl)hydrazinyl}-3-oxopropyl]carbamate (13c)*

Yield of **13c**: 34%; Yellow solid; mp 175-177 °C; FTIR (neat, *v*/cm^{-1}): 3387 (NH), 3318 (NH), 3298 (NH), 1684 (C=O), 1653 (amide I), 1647 (C=O), 1638 (amide II), 1578 (C=C), 1541 (C=C), 1521 (C=C), 1508 (C=C); ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ: 1.45 (9H, s, $C(CH_3)_3$), 2.67 (2H, t, *J* = 6.8 Hz, CH_2-α), 3.44 (2H, t, *J* = 6.8 Hz, CH_2-β), 4.56 (1H, s, carbamate NH), 5.32 (2H, s, CH_2), 7.11 (1H, d, *J* = 8.4 Hz, H-5), 7.19-7.23 (1H, m, H-4′), 7.27-7.31 (2H, m, H-3′, 5′), 7.44-7.47 (2H, m, H-2′, 6′), 7.58 (1H, dd, *J* = 8.4, 7.2 Hz, H-8), 8.35 (1H, d, *J* = 8.4 Hz, H-4), 8.43 (1H, dd, *J* = 7.2, 0.8 Hz, H-9), 8.54 (1H, dd, *J* = 8.4, 0.8 Hz, H-7), 8.89 (1H, broad s, amide NH), 9.54 (1H, broad s, amide NH); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ: 29.7 (3 × CH_3, $C(CH_3)_3$), 35.9 (CH_2, CH_2-α), 38.5 (CH_2, CH_2-β), 44.7 (CH_2), 79.9 (quat., $C(CH_3)_3$), 107.7 (CH, C-5), 114.4 (quat., C-3a), 121.4 (quat., C-6a), 124.5 (quat., C-9a), 127.0 (CH, C-8), 128.8 (CH, C-4′), 129.3 (CH, C-7), 130.01 (2 × CH, C-2′, 6′), 130.04 (2 × CH, C-3′, 5′), 131.1 (quat., C-3b), 132.6 (CH, C-9), 135.2 (CH, C-4), 140.2 (quat., C-1′), 152.3 (quat., C-6), 165.1 (C=O, C-3), 165.7 (C=O, C-1), 174.1 (C=O, amide CO); ESI-MS = 511 (M + Na).

2.7 General procedure for the removal of Boc protecting group to give salts 12a-e and 14a-c

Boc-protected substrates **11a-e** and **13a-c** were dissolved in DCM (5 mL) and the solution cooled to 0 °C in an ice bath. DCM / TFA (5 drops of 1:1 mixture) was added to this solution which was allowed to stir for 2 hours while warming to room temperature. The solvent and excess TFA were removed *in vacuo* after the addition of methanol (50 mL). The residue was then triturated with diethyl ether and the solid formed was filtered to give the products **12a-e**, or the reaction vessel was cooled at -80 °C overnight to give the final substrate **14a-c** as a TFA salt.

*2.7.1 3-Amino-*N*-{5-(*N′*-propylsulfamoyl)naphthalen-1-yl}propanamide trifluoroacetate salt (12a)*

Yield of **12a**: 87%; Off-white solid; mp 155-158 °C; FTIR (neat, *v*/cm^{-1}): 3265 (NH), 3163 (NH), 1668 (C=O, amide), 1597 (C=C, aromatic), 1305 (S=O), 1134 (S=O); ^{1}H NMR (400 MHz, D_2O) δ: 0.68 (3H, t, *J* = 7.4 Hz, CH_3), 1.32 (2H, sextet, *J* = 7.2 Hz, CH_2-2′), 2.86 (2H, t, *J* = 6.8 Hz, CH_2-1′), 3.09 (2H, t, *J* = 6.6 Hz, CH_2-α), 3.41 (2H, t, *J* = 6.0 Hz, CH_2-β), 7.65-7.71 (2H, m), 7.78 (1H, t, *J* = 8.2 Hz), 8.26-8.30 (2H, m), 8.58 (1H, d, *J* = 8.8 Hz); ^{13}C NMR (100 MHz, D_2O) δ: 10.2 (CH_3-3′), 22.0 (CH_2, C-2′), 31.9 (CH_2, C-α), 35.4 (CH_2, C-β), 44.45 (CH_2, C-1′), 123.4 (CH), 125.0 (CH), 125.4 (CH), 128.1 (CH), 128.2 (quat.), 128.8 (CH), 129.9 (CH), 130.0 (quat.), 132.5 (quat.), 133.8 (quat.), 172.3 (C=O, amide CO); ESI-MS = 336 (M + H); HRMS *Calcd. for* $C_{16}H_{22}N_3O_3S$: 336.1376. Found: 336.1379.

*2.7.2 3-Amino-*N*-{5-(*N′*-butylsulfamoyl)naphthalen-1-yl}propanamide trifluoroacetate salt (12b)*

Yield of **12b**: 53%; Off-white solid; mp 152-155 °C; FTIR (neat, *v*/cm^{-1}): 3267 (NH), 1670 (C=O, amide), 1597 (C=C, aromatic), 1319 (S=O), 1134 (S=O); ^{1}H NMR (400 MHz, D_2O) δ: 0.60 (3H, t, *J* = 7.4 Hz, CH_3), 1.06 (2H, sextet, *J* = 7.4 Hz, CH_2-3′), 1.235 (2H, quintet, *J* = 7.2 Hz, CH_2-2′), 2.88 (2H, t, *J* = 6.8 Hz, CH_2-1′), 3.08 (2H, t, *J* = 6.5 Hz, CH_2-α), 3.40 (2H, t, *J* = 6.5 Hz, CH_2-β), 7.63-7.68 (2H, m), 7.76 (1H, t, *J* = 8.2 Hz), 8.24-8.27 (2H, m), 8.56 (1H, d, *J* = 8.8 Hz); ^{13}C NMR (100MHz, D_2O) δ: 12.4 (CH_3), 18.8 (CH_2, C-3′), 30.4 (CH_2, C-2′), 31.9 (CH_2, C-α), 35.4 (CH_2, C-β), 42.2 (CH_2, C-1′), 123.3 (CH), 125.0 (CH), 125.3 (CH), 128.1 (CH), 128.2 (quat.), 128.8 (CH), 130.0 (CH and quat.), 132.5 (quat.), 133.7 (quat.), 172.3 (C=O, amide CO); ESI-MS = 350 (M + H); HRMS *Calcd. for* $C_{17}H_{24}N_3O_3S$: 350.1533. Found: 350.1536.

*2.7.3 3-Amino-*N*-{5-(*N′*-pentylsulfamoyl)naphthalen-1-yl}propanamide trifluoroacetate salt (12c)*

Yield of **12c**: 95%; White solid; mp 141-144 °C; FTIR (neat, *v*/cm^{-1}): 3294 (NH), 3265 (NH), 1670 (C=O, amide), 1624 (C=C, aromatic), 1290 (S=O), 1125 (S=O); ^{1}H NMR (400 MHz, D_2O) δ: 0.53 (3H, t, *J* = 6.4 Hz, CH_3), 0.84-0.97 (4H, m, 2 × CH_2, CH_2-3′,4′), 1.19 (2H, t, *J* = 6.6 Hz, CH_2-2′), 2.86 (2H, t, *J* = 6.6 Hz, CH_2-1′), 3.07 (2H, t, *J* = 6.6 Hz, CH_2-α), 3.40 (2H, t, *J* = 6.4 Hz, CH_2-β), 7.60-7.64 (2H, m), 7.74 (1H, t, *J* = 7.6 Hz), 8.20-8.245 (2H, m), 8.54 (1H, d, *J* = 8.8 Hz); ^{13}C NMR (100 MHz, D_2O) δ: 12.8 (CH_3), 21.05 (CH_2, C-4′), 27.6 (CH_2, C-3′), 27.85 (CH_2, C-2′), 32.0 (CH_2, C-α), 35.4 (CH_2, C-β), 42.4 (CH_2, C-1′), 123.3 (CH), 124.95 (CH), 125.2 (CH), 128.0 (CH), 128.15 (quat.), 128.7 (CH), 129.8 (quat.), 129.9 (CH), 132.5 (quat.), 133.8 (quat.), 172.2 (C=O, amide CO); ESI-MS = 364 (M + H); HRMS *Calcd. for* $C_{18}H_{26}N_3O_3S$: 364.1689. Found: 364.1692.

*2.7.4 3-Amino-*N*-{5-(*N′*-octylsulfamoyl)naphthalen-1-yl}propanamide trifluoroacetate salt (12d)*

Yield of **12d**: 58%; Off-white solid; 175-177 °C; FTIR (neat, *v*/cm^{-1}): 3250 (NH), 1668 (C=O, amide), 1599 (C=C, aromatic), 1314 (S=O), 1132 (S=O); ^{1}H NMR (400 MHz, D_2O) δ: 0.67 (3H, t, *J* = 7.2 Hz, CH_3), 0.80-1.11 (10H, m, 5 × CH_2), 1.20 (2H, br s, CH_2-2′), 2.70 (2H, br s, CH_2-1′), 2.91 (2H, t, *J* = 6.4 Hz, CH_2-β), 3.24 (2H, t, *J* = 6.4 Hz, CH_2-α), 7.33-7.44 (3H, m), 7.94 (1H, d, *J* = 6.8 Hz), 8.09 (1H, d, *J* = 8.4 Hz), 8.40 (1H, d, *J* = 8.0 Hz); ^{13}C NMR (100 MHz, D_2O) δ: 13.5 (CH_3), 22.2 (CH_2), 26.0 (CH_2), 28.5 (CH_2), 28.7 (CH_2), 29.1 (CH_2-2′), 31.3 (CH_2), 32.0 (CH_2-β), 35.3 (CH_2-α), 42.6 (CH_2-1′), 123.0 (CH), 124.5 (2 × CH), 127.75 (CH), 128.1 (quat.), 128.5 (CH), 129.0 (CH), 129.45 (quat.), 132.5 (quat.), 134.4 (quat.), 171.6 (C=O, amide CO); ESI-MS = 406 (M + H); HRMS *Calcd. for* $C_{21}H_{32}N_3O_3S$: 406.2159. Found: 406.2161.

*2.7.5 3-Amino-*N*-{5-(*N′*-benzylsulfamoyl)naphthalen-1-yl}propanamide trifluoroacetate salt (12e)*

The reaction of 5-amino-*N*-propylnaphthalene-1-sulfonamide **7e** (0.55 g, 1.76 mmol) with Boc-β-alanine (2.665 g, 14.08 mmol), DCCI (1.451 g, 7.04 mmol), EDIPA (0.273 g, 2.11 mmol) and DMAP (0.129 g, 1.06 mmol), as described above, gave a white solid **11e** (1.038 g) which was used without further purification.

Carbamate **11e** (0.9g) was deprotected using the general method to give a beige solid which was taken up in water, then the pH was adjusted to 14 with 1M aq. NaOH (aq). The aqueous layer was then extracted with ethyl acetate (5 × 30 mL), and the combined organic layers were dried over Na_2SO_4 and reduced *in vacuo* to give the free amine of amide **12e** (0.49g) as

a white solid. This free base was then converted into the trifluoroacetate salt with TFA (0.15 mL) in DCM (10 mL) at 0°C. The white solid product **11e** was obtained after trituration with diethyl ether (0.438g, 50% over 2 steps); mp 100-102 °C; FTIR (neat, *ν*/cm^{-1}): 3273 (NH), 1655 (C=O, amide), 1599 (C=C, aromatic), 1311 (S=O), 1138 (S=O); ^{1}H NMR (400 MHz, D_2O) δ: 3.06 (2H, t, *J* = 6.6 Hz, CH_2-α), 3.395 (2H, t, *J* = 6.6 Hz, CH_2-β), 4.08 (2H, s, NCH_2), 6.83-6.98 (5H, m, CH_{Ph}), 7.49-7.58 (2H, m), 7.67 (1H, t, *J* = 8.0 Hz), 8.06-8.12 (2H, m), 8.41 (1H, d, *J* = 8.8 Hz); ^{13}C NMR (100 MHz, D_2O) δ: 31.9 (CH_2, CH_2-α), 35.4 (CH_2, CH_2-β), 46.2 (NCH_2), 123.1 (CH), 124.9 (CH), 125.0 (CH), 127.2 (C-4′), 127.4 (C-2′ and C-6′), 127.8 (C-3′ and C-5′), 127.9 (CH), 128.0 (quat.), 128.7 (CH), 129.6 (quat.), 130.2 (CH), 132.3 (quat.), 133.8 (quat.), 135.5 (C-1′), 172.1 (C=O, amide CO); ESI-MS (ESI) = 384 (M + H); HRMS *Calcd. for* $C_{20}H_{22}N_3O_3S$: 384.1376. Found: 384.1379.

*2.7.6 3-Amino-*N′*-(2-propyl-1,3-dioxo-2,3-dihydro-1*H*-benz[*de*]isoquinolin-6-yl)propanehydrazide trifluoroacetate (14a)*

Yield of **14a**: 89%; Light orange crystals; mp 134-136 °C; FTIR (nujol, *ν*/cm^{-1}): 3482 (NH), 3252 (NH), 1687 (C=O), 1672 (amide I), 1646 (C=O), 1614 (amide II), 1582 (C=C), 1533 (C=C); ^{1}H NMR (400 MHz, CD_3OD) δ: 0.99 (3H, t, *J* = 7.6 Hz, C-3′), 1.73 (2H, sextet, *J* = 7.6 Hz, C-2′), 2.86 (2H, t, *J* = 6.8 Hz, CH_2-α), 3.31-3.34 (2H, m, CH_2-β), 4.07-4.11 (2H, m, H-1′), 7.03 (1H, d, *J* = 8.4 Hz, H-5), 7.70-7.74 (1H, m, H-8), 8.40 (1H, d, *J* = 8.4 Hz, H-4), 8.51 (1H, d, *J* = 8.8 Hz, H-9), 8.55 (1H, d, *J* = 7.6 Hz, H-7); ^{13}C NMR (100 MHz, CD_3OD) δ: 11.8 (CH_3, C-3′), 22.5 (CH_2, C-2′), 31.3 (CH_2, CH_2-α), 36.8 (CH_2, CH_2-β), 42.7 (CH_2, C-1′), 106.5 (CH, C-5), 113.4 (quat., C-3a), 120.9 (quat., C-6a), 123.7 (quat., C-9a), 126.4 (CH, C-8), 129.1 (CH, C-7), 130.7 (quat., C-3b), 132.4 (CH, C-9), 135.0 (CH, C-4), 151.7 (quat., C-6), 165.7 (C=O, C-3), 166.0 (C=O, C-1), 172.3 (C=O); ESI-MS = 341 (M + H); HRMS *Calcd. for* $C_{18}H_{21}N_4O_3$: 341.1608. Found: 341.1605.

*2.7.7 3-Amino-*N′*-(2-pentyl-1,3-dioxo-2,3-dihydro-1*H*-benz[*de*]isoquinolin-6-yl)propanehydrazide trifluoroacetate (14b)*

Yield of **14b**: 94%; Bright yellow solid; mp 122-127 °C; FTIR (nujol, *ν*/cm^{-1}): 3392 (NH), 3336 (NH), 3197 (NH), 1707 (C=O), 1674 (amide I), 1638 (C=O), 1615 (amide II), 1585 (C=C), 1532 (C=C); ^{1}H NMR (400 MHz, CD_3OD) δ: 0.92 (3H, t, *J* = 6.8 Hz, H-5′), 1.35-1.40 (4H, m, H-3′, H-4′), 1.68 (2H, quintet, *J* = 7.6 Hz, H-2′), 2.85 (2H, t, *J* = 6.4 Hz, CH_2-α), 3.29 (2H, m, CH_2-β), 4.08 (2H, t, *J* = 7.6 Hz, H-1′), 7.00 (1H, d, *J* = 8.4 Hz, H-5), 7.66 (1H, dd, *J* = 8.4, 7.2 Hz, H-8), 8.36 (1H, d, *J* = 8.4 Hz, H-4), 8.46 (1H, dd, *J* = 8.4, 0.8 Hz, H-9), 8.49

(1H, dd, *J* = 7.2, 0.8 Hz, H-7); ^{13}C NMR (100 MHz, CD_3OD) δ: 14.4 (CH_3, C-5′), 23.5 (CH_2, C-4′), 28.9 (CH_2, C-2′), 30.4 (CH_2, C-3′), 31.2 (CH_2, CH_2-α), 36.8 (CH_2, CH_2-β), 41.2 (CH_2, C-1′), 106.5 (CH, C-5), 113.4 (quat., C-3a), 120.9 (quat., C-6a), 123.7 (quat., C-9a), 126.4 (CH, C-8), 129.1 (CH, C-7), 130.7 (quat., C-3b), 132.4 (CH, C-9), 134.9 (CH, C-4), 151.7 (quat., C-6), 165.6 (C=O, C-3), 166.0 (C=O, C-1), 172.3 (C=O); ESI-MS = 369 (M + H); HRMS *Calcd. for* $C_{20}H_{25}N_4O_3$: 369.1921. Found: 369.1918.

*2.7.8 3-Amino-*N′*-(2-benzyl-1,3-dioxo-2,3-dihydro-1*H*-benz[*de*]isoquinolin-6-yl)propanehydrazide trifluoroacetate (14c)*

Yield of **14c**: 96%; Orange solid; mp 131-133 °C; FTIR (nujol, *ν*/cm^{-1}): 3462 (NH), 3219 (NH), 1701 (C=O), 1684 (amide I), 1640 (C=O), 1614 (amide II), 1577 (C=C); ^{1}H NMR (400 MHz, CD_3OD) δ: 2.86 (2H, t, *J* = 6.8 Hz, CH_2-α), 3.30-3.35 (2H, m, CH_2-β), 5.32 (2H, s, CH_2), 7.03 (1H, d, *J* = 8.4 Hz, H-5), 7.19-7.23 (1H, m, H-4′), 7.26-7.30 (2H, m, H-3′, 5′), 7.40-7.42 (2H, m, H-2′, 6′), 7.72 (1H, dd, *J* = 8.4, 7.6 Hz, H-8), 8.41 (1H, d, *J* = 8.4 Hz, H-4), 8.52 (1H, dd, *J* = 8.4, 0.8 Hz, H-9), 8.56 (1H, dd, *J* = 7.6, 0.8 Hz, H-7); ^{13}C NMR (100 MHz, (CD_3OD) δ: 31.2 (CH_2, CH_2-α), 36.8 (CH_2, CH_2-β), 44.3 (CH_2), 106.6 (CH, C-5), 113.2 (quat., C-3a), 120.9 (quat., C-6a), 123.6 (quat., C-9a), 126.5 (CH, C-8), 128.3 (CH, C-4′), 129.2 (2 × CH, C-2′, 6′), 129.3 (CH, C-7), 129.4 (2 × CH, C-3′, 5′), 130.8 (quat., C-3b), 132.6 (CH, C-9), 135.2 (CH, C-4), 139.1 (quat., C-1′), 151.8 (quat., C-6), 165.6 (C=O, C-3), 166.0 (C=O, C-1), 172.3 (C=O); ESI-MS = 389 (M + H); HRMS *Calcd. for* $C_{22}H_{21}N_4O_3$: 389.1608. Found: 389.1606.

2.8 General method for the coupling of hydrazines 10 to aryl aldehydes

The hydrazines **10a-c** (1 equiv.) and arylaldehydes (1.1 equiv.) were dissolved in ethanol (25 mL) and stirred at 80 °C for 2 hours with five drops of glacial acetic acid as a catalyst. The solution was then allowed to cool and the formed precipitate was taken to obtain final compounds **17-21**.

2.9 Fluorescence properties

In vitro fluorescent assessment of fluorophores (**7a-e**: 5 × 10^{-4} M; **10a-c**: 1 × 10^{-4} M), substrates (**12a-e**: 5 × 10^{-4} M; **14a-c**: 1 × 10^{-4} M), and hydrazones **17-21** (**17,18**: 1 × 10^{-5} M; **19-21**: 1 × 10^{-4} M) in ethanol was carried out on a Shimadzu RF-5301PC spectrophotometer using identical scan parameters, utilising a 5 mm slit width, if not stated otherwise.

2.10 Microbiological testing

2.10.1 Preparation of culture media containing substrates 12a-e and 14a-c

Columbia agar was prepared as follows; 41 g of Columbia agar (Oxoid Basingstoke, UK) was added to deionised water and the volume was made up to 1 L. The medium was sterilised by autoclaving at 116 °C for 20 minutes and left to cool at 50 °C. 2 mg of each substrate **12a-e** and **14a-c** to be tested was initially dissolved in 100 μL of *N*-methylpyrrolidone and this was added to Columbia agar (made up to 20 mL), then poured into sterile Petri dishes to give a final concentration of 100 mg/L for the substrates. Columbia agar incorporating an equivalent concentration of *N*-methylpyrrolidone was used as a growth control.

2.10.2 Microbial suspension preparation

Microbial reference strains were obtained from either the National Collection of Type Cultures (NCTC) or the National Collection of Pathogenic Fungi (NCPF) which are both located at the Central Public Health England Laboratory, Colindale, UK or the American Type Culture Collection (ATCC), Manassas, USA. The 20 test microorganisms were maintained on Columbia agar.

2.10.3 Multipoint inoculation

Colonies of each microbial strain were harvested using a loop from overnight cultures on Columbia agar. These were suspended in sterile deionised water to a suspension equivalent to 0.5 McFarland units using a densitometer. 100 μL of this suspension was pipetted into the corresponding wells of a multipoint inoculation device. Each set of plates received 1 μL of bacterial suspension, giving 1.5×10^5 organisms per spot on each inoculation. Twenty strains were inoculated per plate and the plates were incubated for 18 hours in air at 37 °C.

2.10.4 Activity determination

After incubation, the activity of the microorganisms with the test substrates was determined by observing the plates under UV irradiation at 365 nm and comparing with the substrate-free control.

3 Results and discussion

3.1 Preparation of 5-amino-1-naphthalenesulfonamides (ANS)

Starting from 5-amino-1-naphthalenesulfonic acid **3**, the amino group was protected via *N*-acetylation as described by Xu *et al.*[18] to give imide **4** in excellent yield, Scheme 1. In order to synthesise the fluorophores **7a-e**, the sulfonic acid group was converted to a sulfonyl chloride **5** [19] which was subsequently reacted with a series of amines, to form the

acetamides **6a-e** in moderate yields *via* a nucleophilic substitution. Removal of the *N*-acetyl group of the acetamides **6a-e** gave the sulfonamide fluorophores **7a-e**.

3.2 Preparation of 6-hydrazinobenz[*de*]isoquinoline-1,3-diones (6-HBI) 10a-c

Synthesis of the desired 6-hydrazinobenz[*de*]isoquinoline-1,3-diones (6-HBI) **10a-c** was achieved in two steps; a range of 6-bromobenz[*de*]isoquinoline-1,3-diones **9a-c** was prepared, in high yields, Scheme 2, from 4-bromo-1,8-naphthalic anhydride **8** by using an adaptation of the method of Wang *et al.*.[20] The subsequent substitution of the bromine was achieved by reaction with hydrazine hydrate in ethanol, under reflux, to give the fluorophores **10a-c**,[14] Scheme 3. This step was optimised *via* the addition of hydrazine in portions over a period of time, resulting in an approximately 20 % increase in reaction yield, and the products were then isolated by recrystallization of the crude residues from 2-butanol.

3.3 Amide 12 and hydrazide 14 formation

Each of the fluorophores **7a-e** and **10a-c** were initially coupled to Boc-β-Ala-OH using the activated pentafluorophenyl ester, as described by Nakatani *et al.*.[21] However, the presumed instability of the ester resulted in low yields of the desired products **11a-e** and **13a-c**. Alternatively, the coupling was carried out by the formation of the symmetrical anhydride of Boc-β-Ala-OH (using DCCI as a dehydrating agent), Scheme 3, which was then added to a mixture of **7a-e** or **10a-c** and DMAP, EDIPA in DCM.[22] The subsequent deprotection of **11a-e** and **13a-c** in TFA / DCM (1:6 v/v) gave the trifluoroacetate salts **12a-e** and **14a-c**, in moderate to good yields, for microbiological evaluation.

3.4 Secondary reaction of 6-hydrazinobenz[*de*]isoquinoline-1,3-diones (6-HBI) 10a-c with aldehydes

A range of aryl aldehydes **15** and **16** and the appropriate 6-hydrazinobenz[*de*]isoquinoline-1,3-dione **10a-c** were stirred under reflux in ethanol for two hours, in the presence of catalytic amounts of glacial acetic acid, Scheme 4. The isolation of products **17-21**, as a range of orange to dark purple solids, was achieved in good yields by the filtration of the precipitate which formed upon cooling.

3.5 Fluorescence properties

As discussed earlier, the fluorescence properties of a substrate compared to those of a fluorophore are sufficient for their application in bacterial diagnostics when a significant change in fluorescence intensity or a significant shift in the emission wavelength is observed.

In order to fully assess the suitability of our core molecules, *in vitro* fluorescent assessment of fluorophores **7a-e**, **10a-c**, substrates **12a-e**, **14a-c**, and hydrazones **17-21** was carried out in ethanol.

3.5.1 Fluorescence properties of ANS derivatives

The ANS substrates **12a-e** displayed excitation maxima of 339 nm across the five compounds while the emission maxima ranged from 421-426 nm, resulting in Stokes shifts of 82-87 nm, Table 1. Changes in the *N*-substituent had little or no effect on the fluorescence properties, as shown by the identical excitation and narrow range of emission maxima. The ANS fluorophores **7a-e**, however, exhibited excitation and emission maxima of 391-408 nm and 514-519 nm, respectively, with Stokes shifts of 111-125 nm as shown in Table 1. Similarly to the substrates, the alkyl chain length of the *N*-substituents had a negligible effect on the observed fluorescence properties, while the benzyl substituent had only a slight effect. When comparing the emission maxima between the substrates **12** and fluorophores **7**, *e.g.* Figure 3, an average bathochromic shift of 93 nm was observed, suggesting that these would be ideal fluorogenic substrates for the detection of bacteria.

3.5.2 Fluorescent properties of 6-HBI derivatives

Evaluation of the fluorescence properties of all synthesized hydrazines **10a-c**, Boc-protected hydrazides **13a-c**, intended enzyme substrates **14a-c** and hydrazones **17-21**, was performed at a concentration of 1×10^{-4} M in ethanol. The hydrazones **17a-c** and **18a-c** displayed significantly enhanced fluorescence, surpassing the detection limit of the spectrophotometer, so that the fluorescence of these compounds was studied at a concentration of 1×10^{-5} M.

Similarly to the ANS derivatives, differences in the *N*-substituents on the 6-HBI core resulted in relatively little effect on the fluorescence properties observed (Figures 4 and 5, Table 2) with the exception of **10c**, where a markedly increased emission intensity was observed compared to derivatives **10a-b**. Interestingly, this increase in fluorescence disappeared once **10c** was coupled to Boc-β-alanine (**13**,**14**) or when reacted with an aldehyde to form hydrazones **17-21**. This decrease in intensity is presumably due, in part, to the engagement of the lone pair of the 4-hydrazinyl moiety in further resonance structures of **13-14** and **17-21**.

The 6-HBI substrates **14a-c** exhibited excitation maxima of 442-444 nm, with corresponding emission maxima of 509-511 nm, with a Stokes shift of 67 nm. In comparision, the fluorophores **10a-c** displayed excitation maxima of 465-476 nm and emission maxima of 534-536 nm. This would correspond to a slight bathochromic shift (23-27 nm) if the

substrates **14a-c** were enzymatically cleaved to give the fluorophores **10a-c**. Unfortunately, such a small shift would be likely produce little or no effect on the observed colour of the fluorescence and a secondary reaction wold need to be utilized to further enhance the differentiation between the fluorophore and the substrate.

Reaction of the fluorophores **10a-c** with aryl aldehydes **15** and **16** resulted in the formation of hydrazones **17-21** with greatly increased fluorescence intensities (2-fold to > 10-fold) in most cases (except for **20a-c**), Figure 5 and Table 3, and a bathochromic shift (compounds **19-21**) of the maximum emission wavelength. The extent of this bathochromic effect on the emission maximum can be related to the electron withdrawing / donating characteristics of the aryl aldehydes used (4-*N*$(CH_3)_2$ > 4-OCH_3 > benzaldehyde > 4-Cl). Electron-donating substituents **19**,**20** displayed greater bathochromic effects (Δ 23-52 nm), while electron-withdrawing substituents **17**,**16** had insignificant effects on the emission wavelengths. Further extension of the conjugation, with an additional double bond, as seen in compounds **21a-c**, resulted in a similar bathochromic effect to that of compounds **19a-c**, containing a weakly electron-donating group.

Hydrazones **20a-c,** containing a dimethylamino group, produced the greatest recorded bacthochromic shift, but were distinguished by relatively weak fluorescence at the studied concentrations (10^{-4} M), presumably due to the facilitated rotational decay to the ground state offered by the dimethylamino group disrupting the electronic properties, and resulting in quenching of the fluorescence.[23] The greatest intensification of fluorescence emission was observed for the benzaldehyde (**17a-c**) and 4-chlorobenzaldehyde (**18a-c**) derived hydrazones.

3.6 Microbiological testing of β-alanylaminonaphthalenesulfonamides (ANS) 12 and 6-(β-alanyl)hydrazinobenz[*de*]isoquinoline-1,3-diones 14

The substrates **12a-e** and **14a-c** were incorporated into a commercially available Columbia medium and subjected to microbiological evaluation against a range of clinically relevant microorganisms. All of the substrates were tested on ten Gram negative and eight Gram positive bacteria and two yeasts.

When observed under long-wave UV light, the colonies of strains hydrolysing the substrates should display a significant change in fluorescence in comparison to those which do not hydrolyse the substrate and the dark background of the medium, Figures 7 and 8. However, none of the visualized plates exhibited the fluorescence associated with the expected released

of fluorophores **7a-e**, Figure 8. Upon further investigation, it was discovered that the medium used for bacterial growth quenches the fluorescence of the released fluorophores **7a-e**, Figure 6.

Furthermore, while substrates **12a-c** and **12e** did not inhibit bacterial growth, in the presence of substrate **12d** only *Providencia rettgeri*, *Serratia marcescens* and *Bukholderia cepacia* were able to grow. The greater toxicity of **12d** compared to the other substrates is presumably due to its longer octyl chain disrupting the integrity of the bacterial cytoplasmic membrane and this is supported by the fact that all three of the surviving microorganisms are known to be resistant to antibacterial agents which target the cell membrane.[24]

Incorporation of substrates **14a-c** into Columbia agar produced a yellow agar with a slightly yellow fluorescent background, Figures 7 and 8. Uptake of the substrates **14a-c** into the bacterial cell greatly increased and localized the fluorescence, which was shown to be suitable for the detection of all bacteria, but it was impossible to differentiate between β-alanyl aminopeptidase producing (spots 5, 7, and 9, Table 4) and non-β-alanyl aminopeptidase producing bacteria. Incorporation of an aryl aldehyde, as previously discussed, would thus be necessary to shift the emission wavelengths to one which is distinct from that of the substrates **10a-c**.

Once again, the substrates **10b** and **10c** containing longer chain lengths inhibited bacterial growth, presumably again due to the disruption of the cell wall. Gram positive and yeast species appeared to be affected more than Gram negative species.

Preliminary data for the inclusion of 4-chlorobenzaldehyde (4-CBA) into growth media showed little to no effect on the observed fluorescence of substrate **14a**, Figure 9. This may be due to a number of factors, such as the lack of enzymatic cleavage of the hydrazide bond to release the fluorophore, lack of reaction between aldehyde and the released hydrazide, failure of the 4-CBA to be taken up into the cell, a reduction in fluorescence emission due to a suboptimal wavelength of 365 nm being used to excite the compounds (λ_{ex} of 465 nm and 467 nm for fluorophore and hydrazone respectively), or a cleavage of the formed hydrazone bond by enzymes expressed by *P. aeruginosa*, similar to results seen by Taniyama *et al.*.[25]

4 Conclusions

A series of novel ANS and 6-HBI fluorophores **7a-e** and **10a-c** were synthesized and were then coupled to β-alanine to form substrates **12a-e** and **14a-c** for β-alanyl aminopeptidase.

Substrates **12d**, **14b** and **14c**, with longer *N*-substituent chains, were associated with growth inhibition of all Gram-positive and a majority of the Gram-negative organisms.

Although preliminary fluorescence data suggested that the ANS fluorophores **9a-e** would display favourable fluorescence characteristics, with a bathochromic shift of ~93 nm from the substrates **12a-e**, as a result of quenching by the medium, Columbia agar incorporating these susbstrates did not appear to selectively differentiate between β-alanyl aminopeptidase producing and non-β-alanyl aminopeptidase producing bacteria. The use of other media or detection formats may, however, alleviate this issue and thus allow for their use in bacterial detection.

The increased fluorescence intensity upon localization of substrate **14a** into all the Gram negative and 7/8 Gram positive bacteria (with the exception of *S. pyogenes*) does, however suggest a use for this substrate when the detection of any bacterial presence is required. For example, as one of the leading causes of sepsis is bacteraemia, a rapid method for the detection of viable bacteria in blood would enable the timely administration of antibacterial agents. This would help to reduce the prevalence of mortality associated with sepsis (10-20 % fatality rate), its progression to severe sepsis (20-50 % fatality rate), and, ultimately, septic shock (40-80 % fatality rate). In the US in the period 1999-2008, the leading bacterial causes of severe sepsis were Gram negative (51.5 %) organisms,[26] with the most common being *E. coli* (20 %), and *Pseudomonas* species (9 %). Gram-positive organisms were responsible for 45.6 % of severe sepsis cases, with methicillin-sensitive *S. aureus* (28 %) being the most common. Of the cases in which *E. coli* was present, 16 % resulted in mortality, while methicillin-sensitive *S. aureus* (MSSA) bacteraemia had a mortality rate of 31 %.[27]

The fluorophore **10a-c** was also reacted with a series of *para*-substituted benzaldehydes and cinnamaldehyde to alter the fluorescence of the fluorophore. Addition of electron-donating *para*-substituted benzaldehydes appeared to form hydrazones **20a-c** with the desired emission wavelengths, although the intensities of emission were severely reduced. Preliminary data with the inclusion of substrate **14a** with 4-chlorobenzaldehyde in Columbia agar showed little to no effect on the observed intensity of fluorescence, and further work must be undetaken to ascertain the mechanism behind these observations. Although the synthesized hydrazones **17-21** did not produce the desired characteristics for incorporation into media, future work should also examine the effects of more strongly electron-donating aldehydes with less rotatable bonds.

Acknowledgements

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Supplementary data

Supplementary data relating to this article can be found at http://

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Figure Legends

Figure 1. Illustration of the principle for the chromogenic / fluorogenic detection of bacteria and the basis of the colour change in chromID *P. aeruginosa*.[7, 8]

Figure 2. Chemical structures and reported excitation and emission wavelengths for core heterocycles, ANS and 6-HBI.

Figure 3. Emission spectra for substrate **12c** (slit width = 3 mm) (dashed blue line) and fluorophore **7c** (slit width = 5 mm) (solid green line) (both 5×10^{-4} M in EtOH) (intensities are shown in arbitrary units).

Figure 4. **a)** Emission spectra (at λ_{ex} = 443 nm) of substrate **14a** in EtOH (1×10^{-4} M) (black line), water (blue line), brine (red line), Mueller-Hinton broth (green line) (100 mg/L in water, brine and Mueller-Hinton broth); **b)** Emission spectra (at λ_{ex} = 465 nm) of fluorophore **10a** in EtOH (1×10^{-4} M) (black line), water (blue line), brine (red line), Mueller-Hinton broth (green line) (100 mg/L in water, brine and Mueller-Hinton broth). The dashed green line in both figures represents the spectrum of Mueller-Hinton broth alone. All iintensities are shown in arbitrary units).

Figure 5. Emission spectra of fluorophore **10a** (dashed blue line) and hydrazones **17a** (solid red line), **18a** (solid green line), **19a** (solid purple line), **20a** (solid black line), and **21a** (solid orange line on baseline) (**17a**, **18a**: 1×10^{-5} M in EtOH, **10a**, **19a**-**21a**: 1×10^{-4} M in EtOH) (slit width = 5 mm) (intensities are shown in arbitrary units).

Figure 6. **a)** Emission spectra (at λ_{ex} = 339 nm) of substrate **12b** in EtOH (5×10^{-4} M) (black line), water (blue line), brine (red line), Mueller-Hinton broth (green line) (100 mg/L in water, brine and Mueller-Hinton broth); **b)** Emission spectra (at λ_{ex} = 394 nm) of fluorophore **7b** in EtOH (5×10^{-4} M) (black line), water (blue line), brine (red line), Mueller-Hinton broth (green line) (100 mg/L in water, brine and Mueller-Hinton broth) The dashed green line in both figures represents the spectrum of Mueller-Hinton broth alone. All iintensities are shown in arbitrary units); **c)** Observed fluorescence (λ_{ex} = 365 nm) of sulfonamide **7b** in Mueller-Hinton broth (left) and ethanol (right).

Figure 7. Culture of various bacterial species with substrates **12a-e** and **14a-c** and control on Columbia agar; inoculated colonies correspond to numbered plate.

Figure 8. Observed fluorescence of various bacterial species with substrates **12a-e** and **14a-c** and control on Columbia agar (λ_{ex} = 365 nm); inoculated colonies correspond to numbered plate.

Figure 9. Observed fluorescence of *Pseudomonas aeruginosa* colonies incubated on Columbia agar in the presence of substrate **14a** (100 mg/L) (left) and substrate **14a** (100 mg/L) along with 4-chlorobenzaldehyde (100 mg/L) (right).

Figure 1

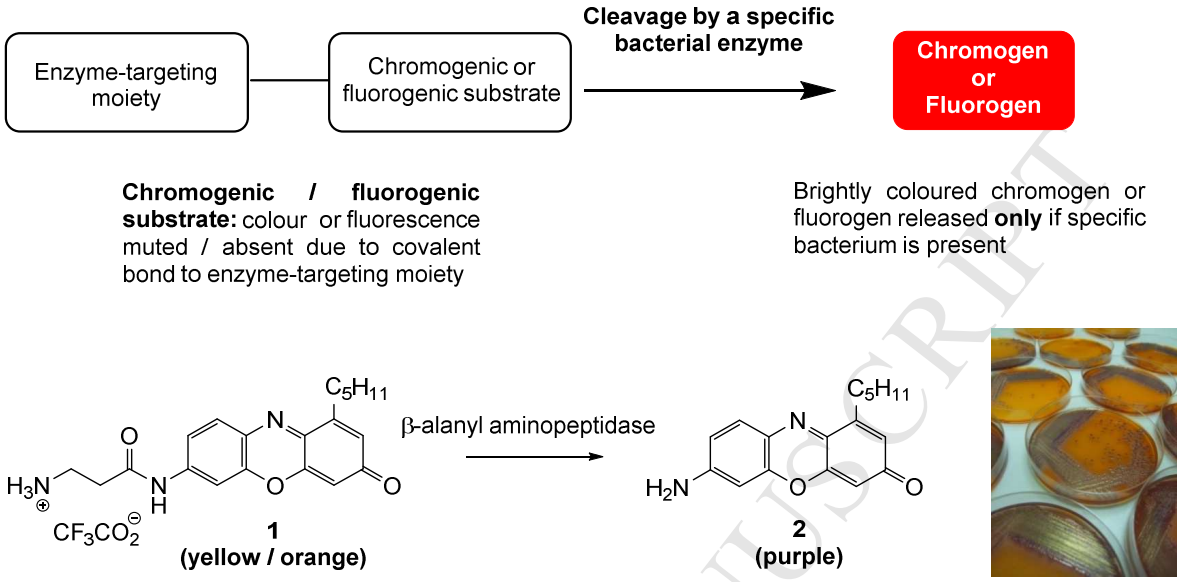
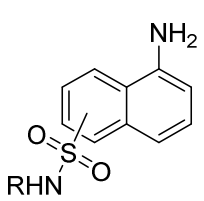
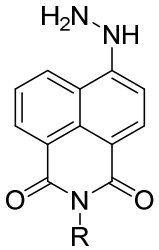


Figure 2





	ANS	**6-HBI**
λ_{ex}	352 nm	330-450 nm
λ_{em}	465-470 nm	370-560 nm

Figure 3

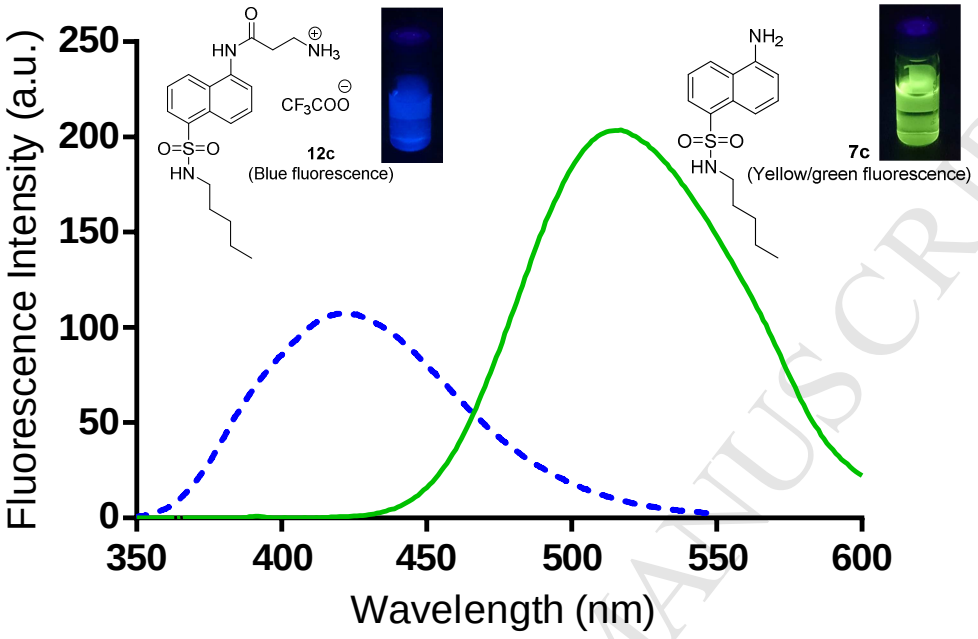
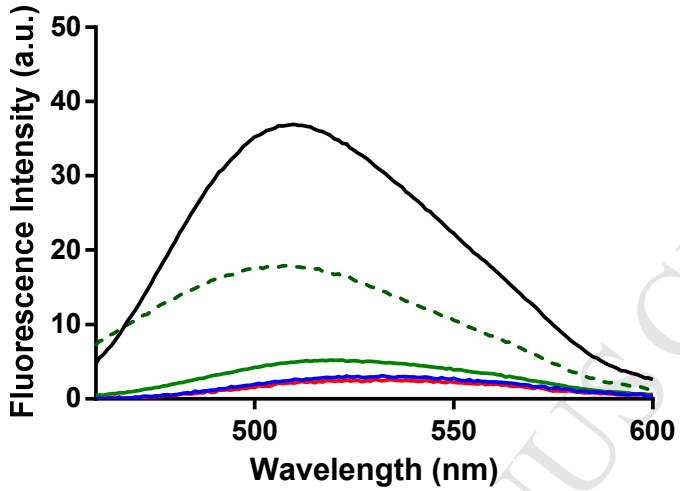


Figure 4



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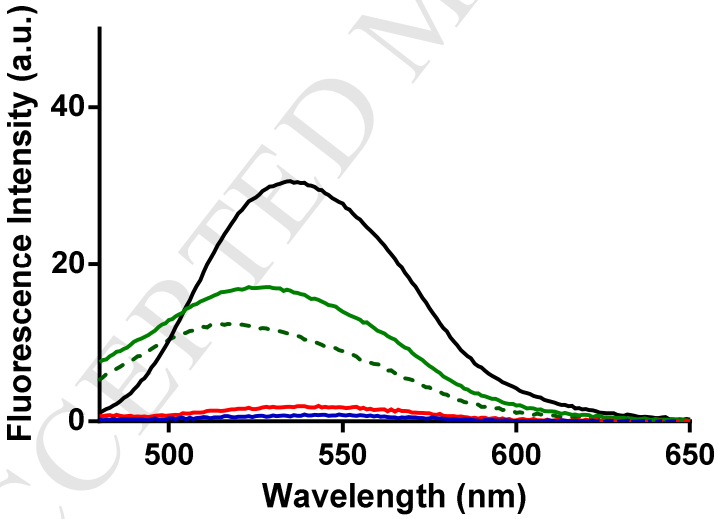


b)

Figure 5

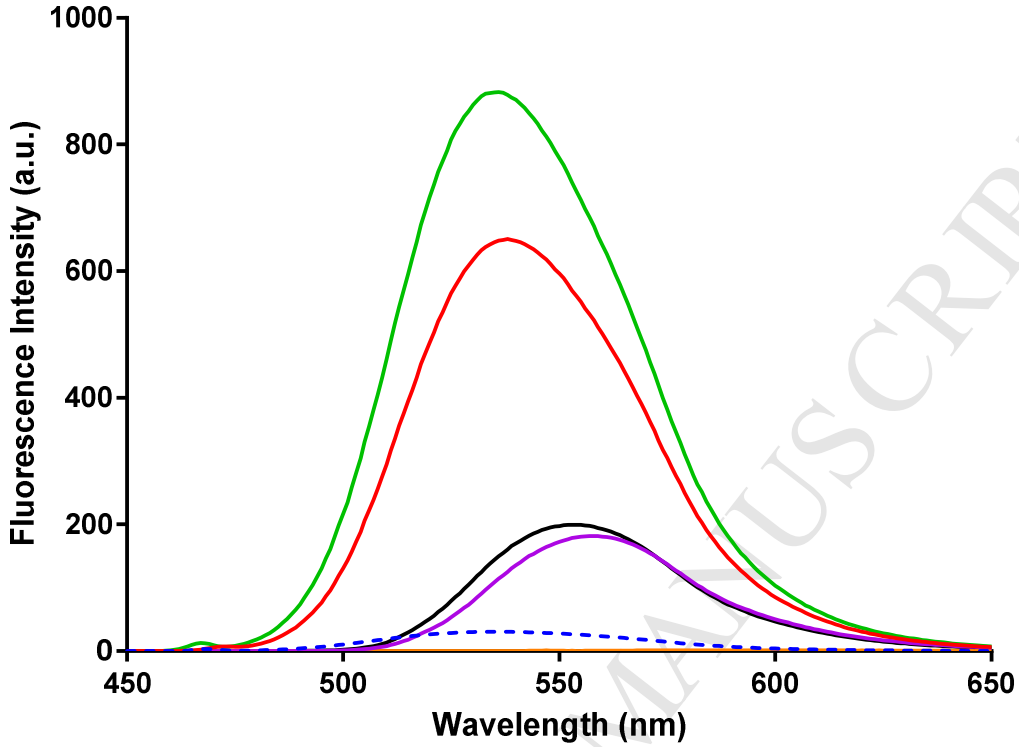
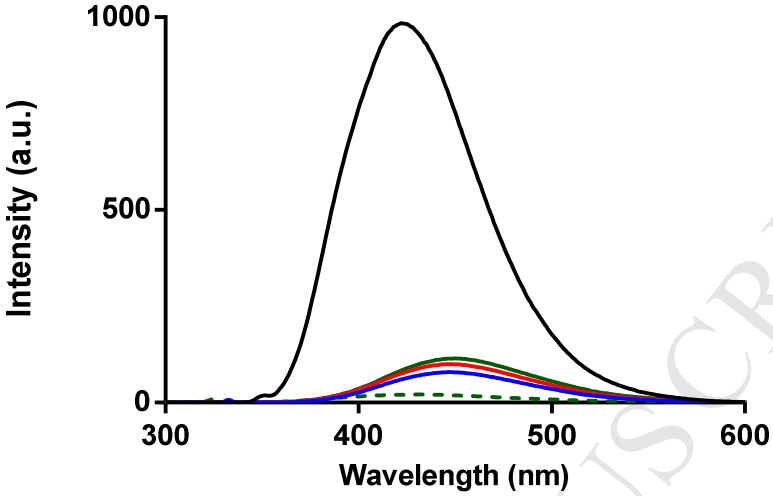


Figure 6



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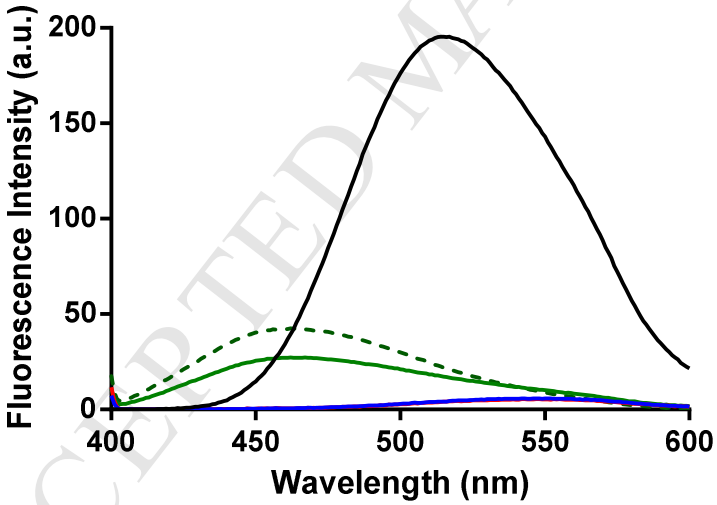


b)

Figure 6 (continued)

c)

Figure 7

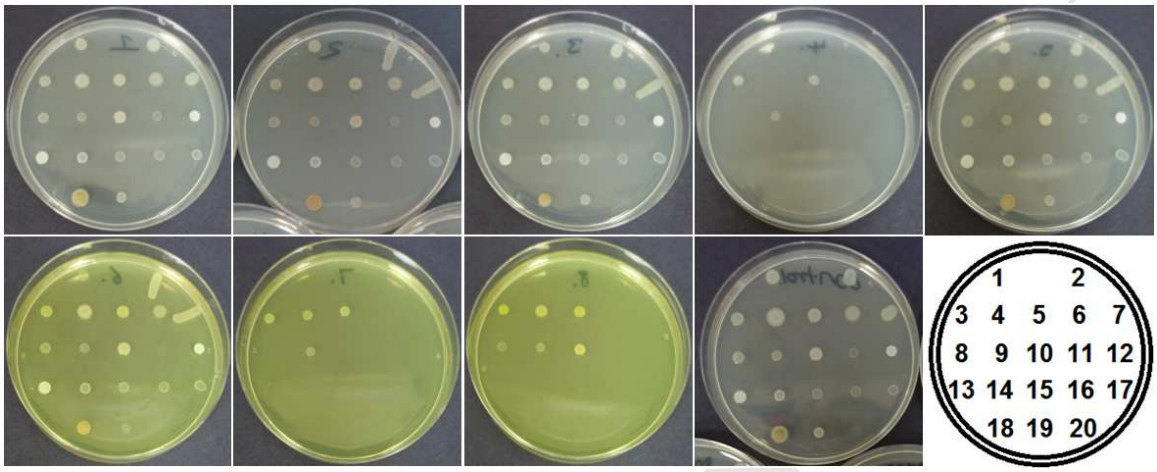


Figure 8

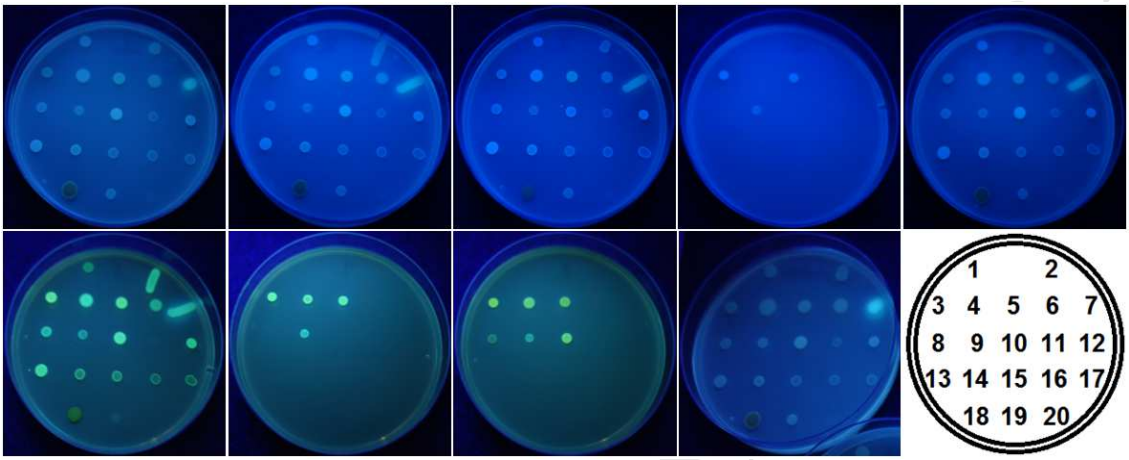
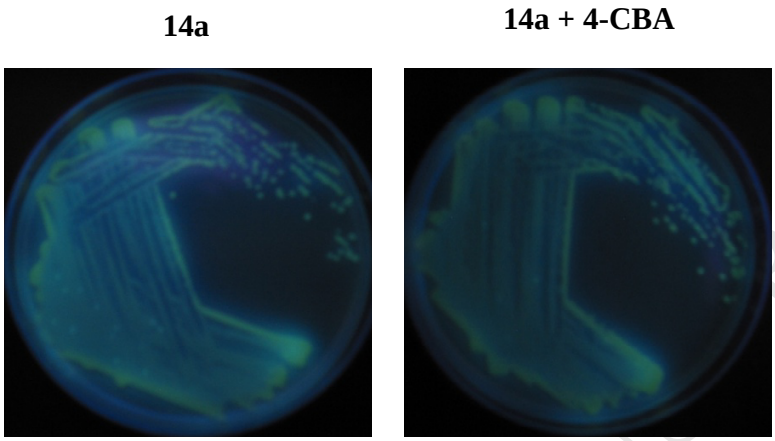


Figure 9



Scheme Legends

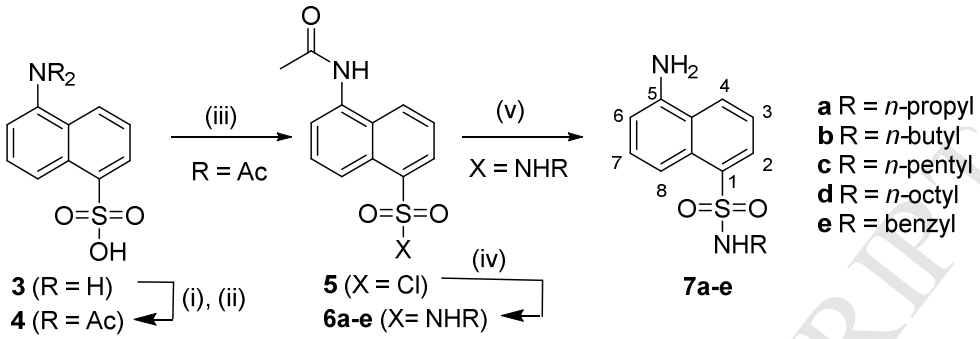
Scheme 1. Synthesis of 5-amino-1-naphthalenesulfonamides **7a-e**. Reagents and conditions: (i) 1M NaOH (aq.), rt, 30 min, 99 %; (ii) Ac_2O, 130 °C, 16 h, 80 %; (iii) $SOCl_2$, DMF, 0 °C to rt, 3 h, 90 %; (iv) RNH_2, Et_3N, DCM, rt, 8 h; (v) 5M NaOH (aq.), 85° C, 20 h.

Scheme 2. Synthesis of 6-hydrazinobenz[*de*]isoquinoline-1,3-diones **10a-c**. Reagents and conditions: (i) RNH_2, EtOH, reflux, 20-25 h; ii) $NH_2NH_2.H_2O$, EtOH, reflux, 24 h.

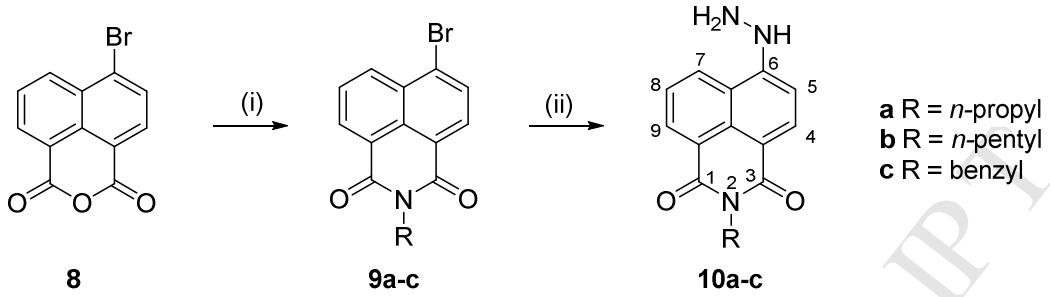
Scheme 3. Amide and hydrazide bond formation and deprotection to give fluorogenic substrates **12a-e** and **14a-c**. Reagents and conditions: (i) Boc-β-Ala-OH, DCC, DIPEA, DMAP, DCM, rt or 50 °C, 24 h; (ii) TFA/DCM (1:6), 0 °C to rt, 2 h.

Scheme 4. Secondary reaction of substituted aryl aldehydes **15** and **16** with 6-hydrazinobenz[*de*]isoquinoline-1,3-diones **10a-c** to form fluorescent hydrazones **17-21**. Reagents and conditions: (i) EtOH, reflux, 2 h.

Scheme 1

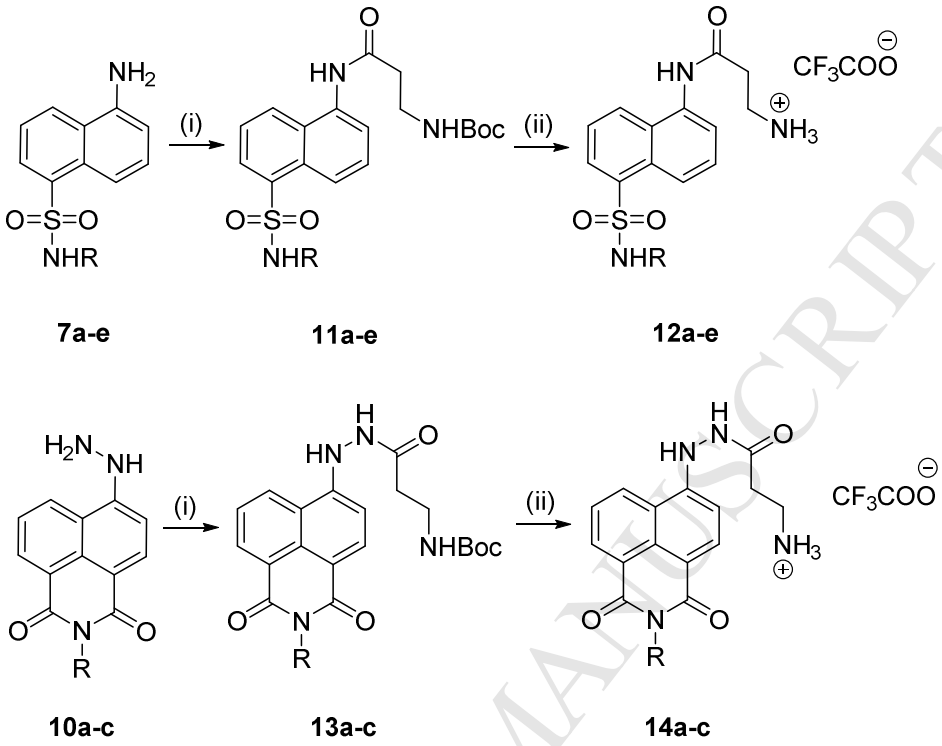


Scheme 2



8 **9a-c** **10a-c**

Scheme 3



Scheme 4

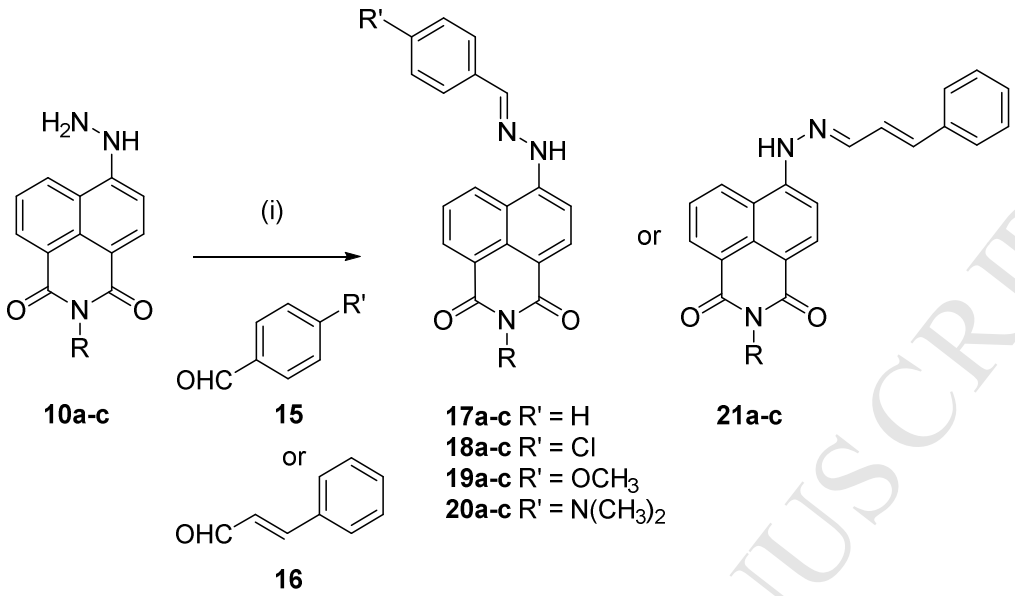


Table Legends

Table 1. Excitation maxima, emission maxima and corresponding Stokes shifts for substrates **12a-e** (slit width = 3 mm) and fluorophores **7a-e** (slit width = 5 mm).

Table 2. Excitation wavelengths, emission wavelengths and the corresponding Stokes shifts of substrates **14a-c** and fluorophores **10a-c**.

Table 3. Excitation wavelengths, emission wavelengths and the corresponding Stokes shifts of hydrazones **17-21**.

Table 4. List of microorganisms inoculated onto incubation plates and corresponding spots (100,000 CFU/spot).

Table 1

Compound		λ_{ex} (nm)	λ_{em} (nm)	Stokes shift (Δν, nm)
12	**a**	339	422	83
	b	339	423	84
	c	339	422	83
	d	339	421	82
	e	339	426	87
7	**a**	391	516	125
	b	394	515	121
	c	392	517	125
	d	395	514	119
	e	408	519	111

Table 2

Compound		λ_{ex}	λ_{em}	Stokes shift (Δν, nm)
	a	443	510	67
14	**b**	444	511	67
	c	442	509	67
	a	465	535	70
10	**b**	476	534	58
	c	472	536	64

Table 3

Compound		λ_{ex}	λ_{em}	Stokes shift (Δν, nm)
	a	467	538	71
17	**b**	469	538	69
	c	472	538	66
	a	467	536	69
18	**b**	467	536	69
	c	471	537	66
	a	517	559	42
19	**b**	519	557	38
	c	517	559	42
	a	433	587	154
20	**b**	424	554	130
	c	433	579	146
	a	516	553	37
21	**b**	516	555	39
	c	519	554	35

Table 4

Spot	Organism	Spot	Organism
1	*Escherichia coli*	**11**	*Steptococcus pyogenes*
2	*Klebsiella pneumoniae*	**12**	*Staphylococcus aureus* (MRSA)
3	*Providencia rettgeri*	**13**	*Staphylococcus aureus*
4	*Enterobacter cloacae*	**14**	*Staphylococcus epidermidis*
5	*Serratia marcescens*	**15**	*Listeria monocytogenes*
6	*Salmonella typhimurium*	**16**	*Enterococcus faecium*
7	*Pseudomonas aeruginosa*	**17**	*Enterococcus faecalis*
8	*Yersinia enterocolitica*	**18**	*Bacillus subtilis*
9	*Burkholderia cepacia*	**19**	*Candida albicans*
10	*Acinetobacter baumannii*	**20**	*Candida glabrata*

- New fluorogenic enzyme substrates prepared by coupling of ANS or HBI with β-alanine
- ANS fluorophores have bathochromic shift of ~93 nm from precursors
- Increased fluorescence intensity on localization of an HBI into bacteria
- Potential secondary reaction products prepared from HBI and aldehydes

Evaluation of fluorogenic aminonaphthalenesulfonamides and 6-hydrazinobenz[*de*]isoquinoline-1,3-diones for the detection of bacteria

Supplementary information

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1. General

All reagents and solvents were purchased from Sigma-Aldrich, Alfa Aesar, and ChemSupply, and used without any further purification or treatment. Thin layer chromatography was performed on Grace Reveleris® Silica Aluminum-backed TLC Plates (UV254). ^{1}H and ^{13}C NMR spectra were acquired on a Varian 400MR at 400 MHz and 100 MHz, respectively. Coupling constants (*J*) are in Hertz (Hz), chemical shifts (δ) are expressed in parts per million (ppm) and reported relative to residual solvent peaks. Melting points were obtained on a Stuart Scientific SMP 10. Low resolution mass spectra were obtained on TSQ Quantum Access Max (Triple Quadrupole) LCMS/MS in positive ion mode. High resolution mass spectra were obtained on a Bruker 7T Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (FTICR) in positive ion mode. Infrared spectra were recorded on a Shimadzu FTIR-8400S and Shimadzu IRTracer-100. Elemental analyses were obtained from the Campbell Microanalytical Laboratory in the University of Otago, NZ.

2. Chemistry

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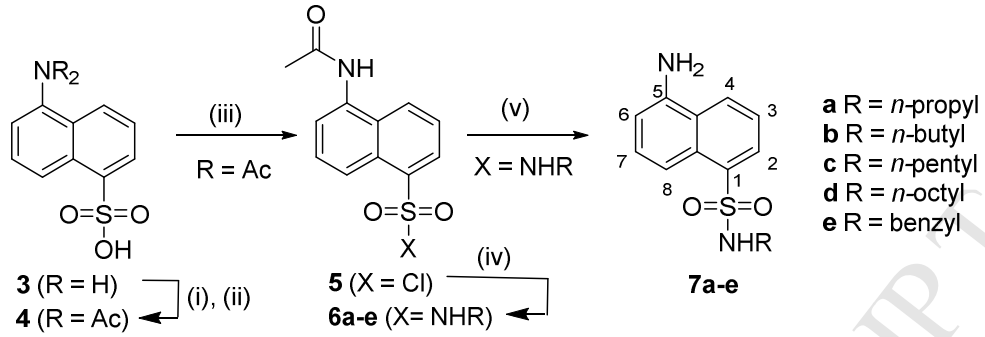
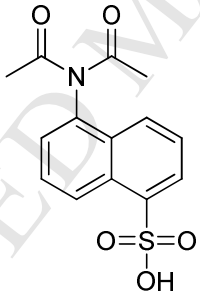


Scheme S1. Synthesis of 5-amino-1-naphthalenesulfonamides **7a-e**. Reagents and conditions: (i) 1M NaOH (aq.), rt, 30 min, 99%; (ii) Ac_2O, 130 °C, 16 h, 80%; (iii) $SOCl_2$, DMF, 0 °C to rt, 3 h, 90%; (iv) RNH_2, Et_3N, DCM, rt, 8 h; (v) 5M NaOH (aq.), 85° C, 20 h.

2.1 Preparation of ANS precursor 5

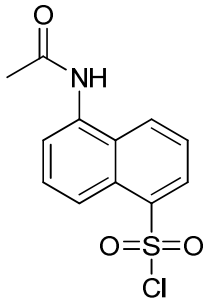
*2.1.1 5-(*N-*Acetylacetamido)naphthalene-1-sulfonic acid (4)* [Xu et al.. Bioorg Med Chem. 2003; 3589-93]



5-Aminonaphthalene-1-sulfonic acid **3** (10.00 g, 44.79 mmol) was dissolved in aqueous NaOH (0.5M, 100 mL) and stirred for 1 hour at room temperature. After removal of the solvent *in vacuo*, the residue was suspended in acetic anhydride (160 mL) and the resulting suspension was refluxed at 140°C for 16 hours. The volatiles were removed *in vacuo* and the crude product was purified by column chromatography on silica, eluting with methanol: DCM (10:90 to 15:85) to give imide **4** as a pale pink solid (12.046 g, 88%); mp ~290 °C decomp.; FTIR (neat, ν_{max}/cm^{-1}): 3489 (OH), 1718 (C=O, imide), 1701 (C=O, imide), 1597 (C=C, aromatic), 1365 (S=O), 1177 (S=O); ^{1}H NMR (400 MHz, CD_3OD) δ: 2.55 (6H, s, 2 × CH_3), 7.51 (1H, dd, *J* = 7.2 Hz, 0.8 Hz), 7.58-7.62 (1H, m), 7.67-7.71 (1H, m), 7.86 (1H, d, *J* = 8.4 Hz), 8.24 (1H, dd, *J* = 7.2 Hz, 0.8 Hz), 9.01 (1H, d, *J* = 8.8 Hz); ^{13}C NMR (100MHz, CD_3OD) δ: 26.57 (2 × CH_3), 125.86 (CH),

127.3 (CH), 127.4 (CH), 127.5 (CH), 128.6 (CH), 129.5 (CH), 131.5 (quat.), 132.6 (quat.), 137.4 (quat.), 143.0 (quat.), 174.9 (C=O, imide CO); ESI-MS = 308 (M + H).

2.1.2 5-Acetamidonaphthalene-1-sulfonyl chloride (5) [Guan et al.. WO/2004/058709, 15 July 2004]

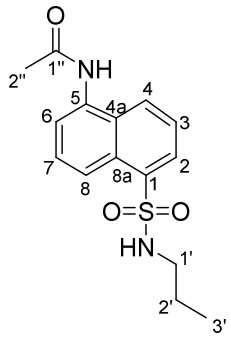


To a solution of imide **4** (9.30 g, 30.26 mmol) in dry DMF (50 mL) at 0 °C, thionyl chloride (2.86 mL, 4.68 g, 39.3 mmol) was added dropwise under a N_2 atmosphere. The resulting solution was then warmed to room temperature and stirred for 2 hours. The reaction mixture was poured over ice-water and the formed precipitate was filtered to yield **5** as a pale beige solid (6.579 g, 77%), which was then used without further purification.

2.2 General procedure for the preparation of acetamides 6a-e

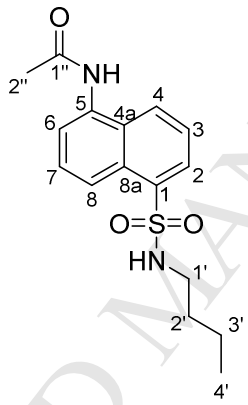
To a solution of **5** (1 equiv.) in DCM, triethylamine (2 equiv.) and the appropriate amine (1.5 equiv.) were added. The resulting solution was then left to stir at room temperature overnight. Upon completion, the organic layer was washed with 1M HCl (3 × 20 mL), water (3 × 20 mL) and brine (3 × 20 mL), then dried over Na_2SO_4. The solvent was removed *in vacuo* and the crude product was purified by column chromatography, eluting with ethyl acetate: hexane (25:75 to 75:25) to give acetamides **6a-e**.

2.2.1 N-*{5-(*N-*Propylsulfamoyl)naphthalen-1-yl}acetamide (6a)*



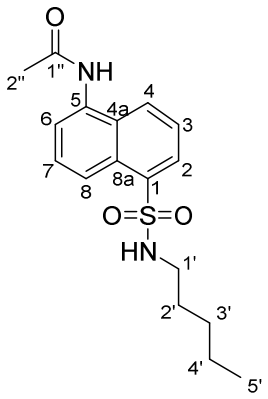
Yield of **6a:** 52%; Beige solid; mp 175-177 °C; FTIR (neat, *v*/cm^{-1}): 3339 (NH), 3227 (NH), 1672 (C=O, amide), 1622 (C=C, aromatic), 1310 (S=O), 1144 (S=O); ^{1}H NMR (400 MHz, CD_3OD) δ: 0.78 (3H, t, *J* = 7.2 Hz, CH_3-3'), 1.37 (2H, sextet, *J* = 7.2 Hz, CH_2-2′), 2.29 (3H, s, CH_3C=O), 2.79 (2H, t, *J* = 7.2 Hz, CH_2-1′), 7.62-7.71 (3H, m), 8.24 (1H, d, *J* = 7.2 Hz), 8.29 (1H, d, *J* = 8.4 Hz), 8.64 (1H, dd, *J* = 7.2 Hz, 2.4 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ: 11.4 (CH_3, C-3′), 23.2 (*C*H_3C=O), 24.0 (CH_2, C-2′), 45.8 (CH_2, C-1′), 124.55 (CH), 125.4 (CH), 125.5 (CH), 128.5 (CH), 129.4 (CH), 130.3 (quat.), 130.4 (CH), 131.3 (quat.), 135.2 (quat.), 137.5 (quat.), 172.95 (C=O, amide CO); ESI-MS = 307 (M + H).

2.2.2 N-*{5-(*N-*Butylsulfamoyl)naphthalen-1-yl}acetamide (6b)*



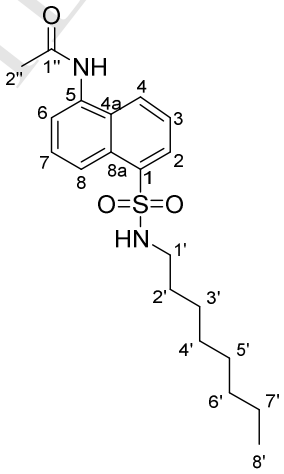
Yield of **6b**; 53%; Beige solid; mp 150-153 °C; FTIR (neat, *v*/cm^{-1}): 3329 (NH), 3215 (NH), 1670 (C=O, amide), 1620 (C=C, aromatic), 1310, 1144 (S=O); ^{1}H NMR (400 MHz, CD_3OD) δ: 0.74 (3H, t, *J* = 7.4 Hz, CH_3-4′), 1.19 (2H, sextet, *J* = 7.4Hz, CH_2-3′), 1.32 (2H, quintet, *J* = 7.2 Hz, CH_2-2′), 2.29 (3H, s, CH_3C=O), 2.82 (2H, t, *J* = 6.8 Hz, CH_2-1′), 7.62-7.71 (3H, m), 8.24 (1H, d, *J* = 7.6 Hz), 8.30 (1H, d, *J* = 8.4 Hz), 8.64 (1H, dd, *J* = 7.6 Hz, 1.6 Hz); ^{13}C NMR (100MHz, CD_3OD) δ: 13.8 (CH_3, C-4′), 20.65 (CH_2, C-3′), 23.2 (*C*H_3C=O), 32.7(CH_2, C-2′), 43.6 (CH_2, C-1′), 124.5 (CH), 125.35 (CH), 125.5 (CH), 128.5 (CH), 129.4 (CH), 130.3 (quat.), 130.4 (CH), 131.3 (quat.), 135.2 (quat.), 137.5 (quat.), 172.9 (C=O, amide CO); ESI-MS = 321 (M + H).

2.2.3 N-*{5-(*N-*Pentylsulfamoyl)naphthalen-1-yl}acetamide (6c)*



Yield of **6c**: 56%; Beige solid; mp 153-157 °C; FTIR (neat, *v*/cm^{-1}): 3337 (NH), 3213 (NH), 1676 (C=O, amide), 1622 (C=C, aromatic), 1307 (S=O), 1140 (S=O); ^{1}H NMR (400 MHz, CD_3OD) δ: 0.73 (3H, t, *J* = 7.0 Hz, CH_3-5′), 1.08-1.13 (4H, m, 2 × CH_2, 2 × H-3′, 2 × H-4′), 1.28-1.35 (2H, m, CH_2-2′), 2.29 (3H, s, CH_3C=O), 2.82 (2H, t, *J* = 7.2 Hz, CH_2-1′), 7.61-7.71 (3H, m), 8.24 (1H, d, *J* = 7.2 Hz), 8.29 (1H, d, *J* = 8.8 Hz), 8.64 (1H, d, *J* = 8.0 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ: 14.2 (CH_3, C-5′), 23.1 (CH_2, C-4′), 23.2 (*C*H_3C=O), 29.7 (CH_2, C-3′), 30.3 (CH_2, C-2′), 43.8 (CH_2, C-1′), 124.5 (CH), 125.3 (CH), 125.5 (CH), 128.5 (CH), 129.4 (CH), 130.3 (quat.), 130.4 (CH), 131.2 (quat.), 135.2 (quat.), 137.5 (quat.), 172.9 (C=O, amide CO); ESI-MS = 335 (M + H).

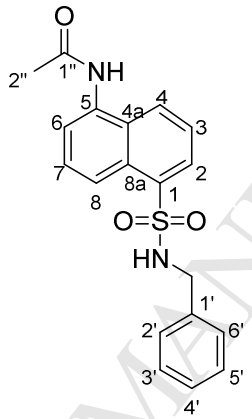
2.2.4 N-*{5-(*N-*Octylsulfamoyl)naphthalen-1-yl}acetamide (6d)*



Yield of **6d**: 29%; Beige solid; mp 142-143 °C; FTIR (neat, *v*/cm^{-1}): 3291 (NH), 3238 (NH), 1655 (C=O, amide), 1622 (C=C, aromatic), 1323 (S=O), 1146 (S=O); ^{1}H NMR (400 MHz, CD_3OD) δ: 0.86 (3H, t, *J* = 7.2 Hz, CH_3-8′), 1.09-1.15 (8H, m, 4 × CH_2), 1.22-1.34 (4H, m, 2 ×

CH_2), 2.29 (3H, s, CH_3C=O), 2.83 (2H, t, *J* = 7.0 Hz, CH_2-1′), 7.62-7.73 (3H, m), 8.24 (1H, d, *J* = 7.2 Hz), 8.30 (1H, d, *J* = 8.4 Hz), 8.64 (1H, d, *J* = 8.0 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ: 14.4 (CH_3, C-8′), 23.2 (CH_3, *C*H_3C=O), 23.7 (CH_2), 27.5 (CH_2), 30.1 (CH_2), 30.2 (CH_2), 30.6 (CH_2), 32.9 (CH_2), 43.9 (CH_2, C-1′), 124.5 (CH), 125.2 (CH), 125.5 (CH), 128.5 (CH), 129.4 (CH), 130.3 (quat.), 130.4 (CH), 131.2 (quat.), 135.25 (quat.), 137.5 (quat.), 172.9 (C=O, amide CO); ESI-MS = 377 (M + H).

2.2.5 N-*{5-(N-Benzylsulfamoyl)naphthalen-1-yl}acetamide (6e)*

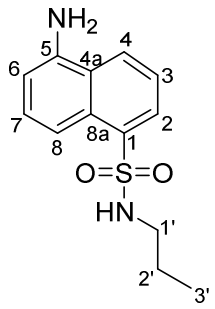


Yield of **6e**: 33%; Beige solid; mp 202-205 °C; FTIR (neat, *ν*/cm^{-1}): 3275 (NH), 1655 (C=O, amide), 1624 (C=C, aromatic), 1306 (S=O), 1140 (S=O); ^{1}H NMR (400 MHz, CD_3OD) δ: 2.30 (3H, s, CH_3), 4.04 (2H, s, NCH_2), 7.03-7.10 (5H, m, CH_{Ph}), 7.56-7.60 (1H, m), 7.64-7.71 (2H, m), 8.21 (1H, m), 8.25 (1H, m), 8.63 (1H, d, *J* = 8.0 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ: 23.2 (CH_3), 47.7 (NCH_2), 124.5 (CH), 125.3 (CH), 125.5 (CH), 128.3 (C-4′), 128.5 (CH), 128.7 (2 CH, C-2′,6′), 129.2 (2 CH, C-3′,5′), 129.4 (CH), 130.2 (CH), 130.5 (CH), 131.2 (CH), 135.2 (CH), 137.6 (CH), 138.4 (C-1′), 172.9 (C=O, amide CO); ESI-MS = 355 (M + H).

2.3 General procedure for the deacetylation of acetamides 6a-e to produce sulfonamides 7a-e

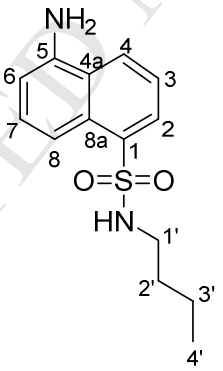
The appropriate acetamide **6a-e** was dissolved in methanol / 5M aqueous NaOH mixture (3:2 v/v) and kept at 85 °C. Upon completion of the reaction, the methanol was removed *in vacuo* and the aqueous residue was extracted with DCM (6 × 25mL). After drying over Na_2SO_4, the organic solvent was removed and the crude product was purified by column chromatography on silica, eluting with ethyl acetate: hexane (50:50 to 60:40) to yield the desired sulfonamides **7a-e**.

*2.3.1 5-Amino-*N*-propylnaphthalene-1-sulfonamide (7a)*



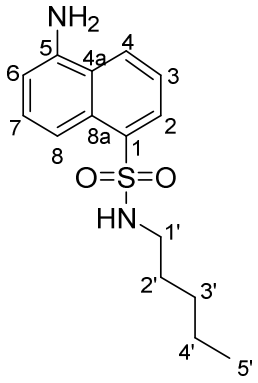
Yield of **7a**: 87%; Yellow solid; mp 117-119 °C; FTIR (neat, *v*/cm^{-1}): 3374 (NH), 3318 (NH), 1638 (C=C, aromatic), 1302 (S=O), 1156 (S=O); ^{1}H NMR (400 MHz, CD_3OD) δ: 0.74 (3H, t, *J* = 7.2 Hz, CH_3), 1.35 (2H, sextet, *J* = 7.2Hz, CH_2-2′), 2.76 (2H, t, *J* = 7.2 Hz, CH_2-1′), 6.92 (1H, d, *J* = 7.5 Hz, H-6), 7.39-7.43 (1H, m, H-7), 7.47-7.50 (1H, m, H-3), 8.00 (1H, d, *J* = 8.6Hz, H-8), 8.15 (1H, d, *J* = 7.3Hz, H-2), 8.31 (1H, d, *J* = 8.6Hz, H-4); ^{13}C NMR (100MHz, CD_3OD) δ: 11.45 (CH_3), 23.95 (CH_2, C-2′), 45.8 (CH_2, C-1′), 111.3 (C-6), 115.2 (C-8), 123.2 (C-3), 125.8 (C-4a), 128.8 (C-4), 129.75 (C-7), 130.1 (C-2), 130.6 (C-8a), 136.6 (C-1), 146.2 (C-5); ESI-MS = 265 (M + H).

2.3.2 5-Amino-N-*butylnaphthalene-1-sulfonamide (7b)*



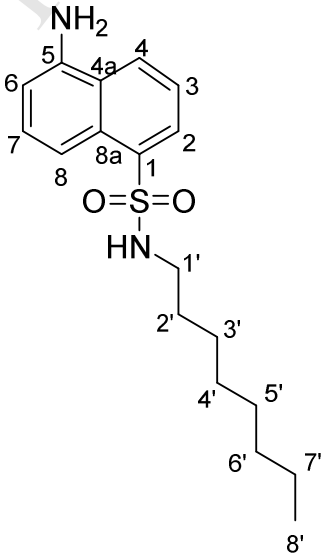
Yield of **7b**: 78%; Yellow solid; mp 116-117°C ; FTIR (neat, *v*/cm^{-1}): 3441, 3370, 3304 (NH), 1637 (C=C, aromatic), 1292, 1150 (S=O); ^{1}H NMR (400 MHz, CD_3OD): δ_H 0.725 (3H, t, *J* = 7.2 Hz, CH_3), 1.17 (2H, sextet, *J* = 7.4 Hz, CH_2-3′), 1.30 (2H, quintet, *J* = 7.2 Hz, CH_2-2′), 2.80 (2H, t, *J* = 6.8 Hz, CH_2-1′), 6.92 (1H, d, *J* = 7.6 Hz, H-6), 7.39-7.43 (1H, m, H-7), 7.46-7.50 (1H, m, H-3), 8.00 (1H, d, *J* = 8.4 Hz, H-8), 8.15 (1H, dd, *J* = 1.0 and 7.4 Hz, H-2), 8.31 (1H, d, *J* = 8.4 Hz, H-4); ^{13}C NMR (100MHz, CD_3OD): δ_C 13.8 (CH_3), 20.6 (CH_2, C-3′), 32.7 (CH_2**,** C-2′), 43.6 (CH_2, C-1′), 111.3 (C-6), 115.2 (C-8), 123.2 (C-3), 125.8 (C-4a), 128.8 (C-4), 129.75 (C-7), 130.1 (C-2), 130.6 (C-8a), 136.6 (C-1), 146.2 (C-5) ; ESI-MS = 279 (M + H).

*2.3.3 5-Amino-*N*-pentylnaphthalene-1-sulfonamide (7c)*



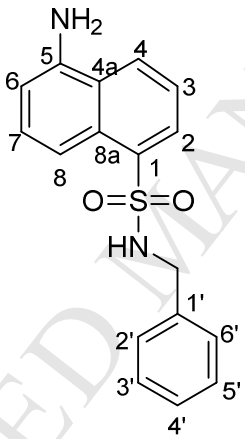
Yield of **7c**: 90%; Yellow solid; mp 97-99°C ; FTIR (neat, v/cm^{-1}): 3390, 3370, 3300 (NH), 1637 (C=C, aromatic), 1298, 1144 (S=O) ;^{1}H NMR (400 MHz, CD_3OD): δ_H 0.72 (3H, t, *J* = 7.0 Hz, CH_3), 1.04-1.13 (4H, m, 2 × CH_2, 2 × H-3′ plus 2 × H-4′), 1.26-1.33 (2H, m, CH_2-2′), 2.80 (2H, t, *J* = 7.0 Hz, CH_2-1′), 6.92 (1H, dd, *J* = 7.6 Hz, 0.8 Hz, H-6), 7.39-7.435 (1H, m, H-7), 7.46-7.50 (1H, m, H-3), 8.00 (1H, d, *J* = 8.8 Hz, H-8), 8.16 (1H, dd, *J* = 1.2 and 7.2 Hz, H-2), 8.31 (1H, d, *J* = 8.4 Hz, H-4);^{13}C NMR (100MHz, CD_3OD): δ_C 14.1 (CH_3), 23.1 (CH_2, C-4′), 29.7 (CH_2, C-3′), 30.2 (CH_2, C-2′), 43.85 (CH_2, C-1′), 111.3 (C-6), 115.2 (C-8), 123.2 (C-3), 125.8 (C-4a), 128.8 (C-4), 129.75 (C-7), 130.1 (C-2), 130.6 (C-8a), 136.7 (C-1), 146.2 (C-5) ; ESI-MS = 293 (M + H).

*2.3.4 5-Amino-*N*-octylnaphthalene-1-sulfonamide (7d)*

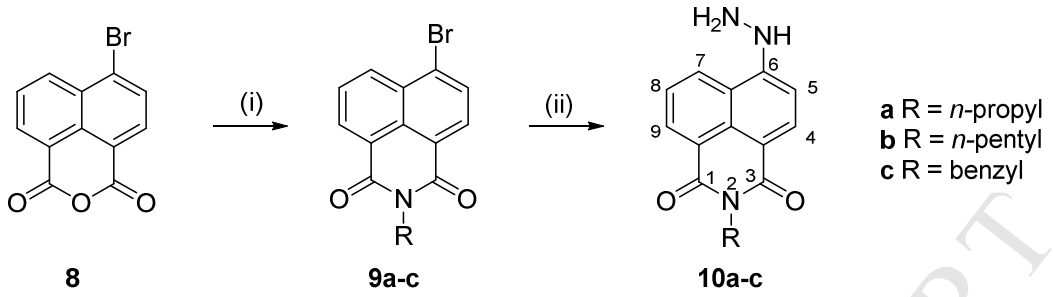


Yield of **7d**: 82%; Yellow solid; mp 78-79°C ; FTIR (neat, v/cm^{-1}): 3423, 3352, 3267 (NH), 1630 (C=C, aromatic), 1296, 1138 (S=O);^{1}H NMR (400 MHz, CD_3OD): δ_H 0.86 (3H, t, *J* = 7.2Hz, CH_3), 0.99-1.16 (8H, m, 4 × CH_2), 1.195-1.31 (4H, m, 2 × CH_2), 2.81 (2H, t, *J* = 7.0Hz, CH_2-1′), 6.91 (1H, d, *J* = 6.8Hz, H-6), 7.39-7.43 (1H, m, H-7), 7.46-7.50 (1H, m, H-3), 7.99 (1H, d, *J* = 8.0Hz, H-8), 8.16 (1H, dd, *J* = 7.4Hz, 1.1 Hz, H-2), 8.32 (1H, d, *J* = 8.8Hz, H-4) ; ^{13}C NMR (100MHz, CD_3OD): δ_C 14.4 (CH_3), 23.7 (CH_2), 27.5 (CH_2), 30.1 (CH_2), 30.2 (CH_2), 30.45 (CH_2), 32.9 (CH_2), 43.8 (CH_2, C-1′), 111.2 (C-6), 115.1 (C-8), 123.2 (C-3), 125.8 (C-4a), 128.8 (C-4), 129.8 (C-7), 130.15 (C-2),130.6 (C-8a), 136.7 (C-1), 146.25 (C-5) ; ESI-MS = 335 (M + H).

2.3.5 5-Amino-N-*benzylnaphthalene-1-sulfonamide (7e)*



Yield of **7e**: 92%; Yellow solid; mp 182-184°C ; FTIR (neat, v/cm^{-1}): 3450, 3375, 3284 (NH), 1634 (C=C, aromatic), 1298, 1152 (S=O) ; ^{1}H NMR (400 MHz, CD_3OD): δ_H 4.00 (NCH_2), 6.91 (1H, s, *J* = 6.8 Hz, H-6), 7.05-7.11 (5H, m, CH_{Ph}), 7.38-7.45 (2H, m, H-3 and H-7), 8.00 (1H, d, *J* = 8.4 Hz, H-8), 8.14 (1H, dd, *J* = 7.4 Hz, 0.8 Hz, H-2), 8.28 (1H, d, *J* = 8.8 Hz, H-4) ; ^{13}C NMR (100MHz, CD_3OD): δ_C 47.7 (CH_2), 111.3 (C-6), 115.2 (C-8), 123.2 (C-3), 125.8 (C-4a), 128.2 (C-4′), 128.7 (C-2′ and C-6′), 128.8 (C-4), 129.1 (C-3′ and C-5′), 129.8 (C-7), 130.2 (C-2), 130.5 (C-8a), 136.6 (C-1), 138.6 (C-1′), 149.2 (C-5) ; ESI-MS = 313 (M + H).



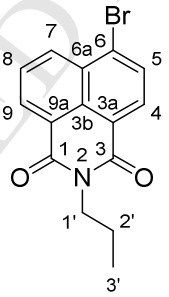
8 **9a-c** **10a-c**

Scheme S2. Synthesis of 6-hydrazinobenz[*de*]isoquinoline-1,3-diones **10a-c**. Reagents and conditions: (i) RNH_2, EtOH, reflux, 20-25 h; ii) $NH_2NH_2.H_2O$, EtOH, reflux, 24 h.

2.4 General method for preparation of 6-bromo-1*H*-benz[*de*]isoquinoline-1,3(2*H*)-diones 9

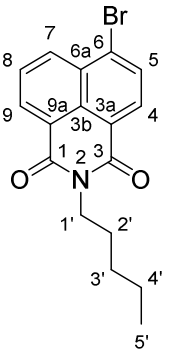
4-Bromo-1,8-naphthalic anhydride **8** (1 equiv.) and the corresponding amine (1 equiv.) were refluxed in ethanol (25 mL) for 2 hours. The solution was then allowed to cool to room temperature and the precipitate which formed was collected by filtration to give the desired product **9a-c**.

*2.4.1 2-Propyl-6-bromo-1*H*-benz[*de*]isoquinoline-1,3(2*H*)-dione (9a)*



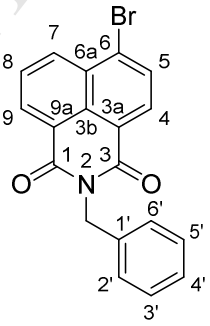
Yield of **9a**: 83%; Brown solid; mp 130-132 °C; FTIR (nujol, ν/cm^{-1}): 1698 (C=O), 1661 (C=O), 1585 (C=C), 1568 (C=C); ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ_H 0.92 (3H, t, J = 7.6 Hz, H-3′), 1.64 (2H, sextet, J = 7.6 Hz, H-2′), 3.97 (2H, t, J = 7.6 Hz, H-1′), 7.92-7.96 (1H, m, H-8), 8.15 (1H, d, J = 8.0 Hz, H-5), 8.26 (1H, d, J = 8.0 Hz, H-4), 8.47 (1H, d, J = 8.4 Hz, H-7), 8.50 (1H, d, J = 7.2 Hz, H-9); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) δ_C 11.3 (CH_3, C-3′), 20.7 (CH_2, C-2′), 41.2 (CH_2, C-1′), 121.8 (quat., C-3a), 122.6 (quat., C-9a), 128.1 (quat., C-3b), 128.6 (CH, C-8), 129.0 (quat., C-6), 129.6 (quat., C-6a), 130.8 (CH, C-4), 131.2 (CH, C-5), 131.4 (CH, C-7), 132.4 (CH, C-9), 162.69 (C=O, C-3), 162.74 (C=O, C-1); ESI-MS = 320, 318 (M + H).

*2.4.2 2-Pentyl-6-bromo-1*H*-benz[*de*]isoquinoline-1,3(2*H*)-dione (9b)*



Yield of **9b**: 62%; Brown crystalline solid; mp 94-96 °C; FTIR (neat, ν/cm^{-1}): 1697 (C=O), 1647 (C=O), 1591 (C=C), 1570 (C=C), 1560 (C=C); ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ_H 0.88 (3H, t, *J* = 7.2 Hz, H-5′), 1.31-1.34 (4H, m, H-3′, H-4′), 1.63 (2H, quintet, *J* = 7.6 Hz, H-2′), 4.02 (2H, t, *J* = 7.6 Hz, H-1′), 7.98-8.02 (1H, m, H-8), 8.23 (1H, d, *J* = 8.0 Hz, H-5), 8.34 (1H, d, *J* = 8.0 Hz, H-4), 8.55-8.59 (2H, m, H-7, H-9); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) δ_C 13.8 (CH_3, C-5′), 21.8 (CH_2, C-4′), 27.0 (CH_2, C-2′), 28.6 (CH_2, C-3′), 39.7 (CH_2, C-1′), 121.9 (quat., C-3a), 122.6 (quat., C-9a), 128.2 (quat., C-3b), 128.7 (CH, C-8), 129.0 (quat., C-6), 129.7 (quat., C-6a), 130.9 (CH, C-4), 131.3 (CH, C-5), 131.5 (CH, C-7), 132.5 (CH, C-9), 162.69 (C=O, C-3), 162.74 (C=O, C-1); ESI-MS = 346, 348 (M + H).

*2.4.3 2-Benzyl-6-bromo-1*H*-benz[*de*]isoquinoline-1,3(2*H*)-dione (9c)*



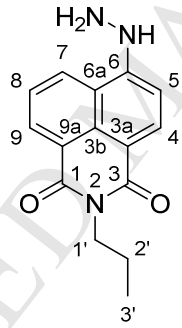
Yield of **9c**: 93%; Brownish white; mp 173-174 °C; FTIR (nujol, ν/cm^{-1}): 1698 (C=O), 1657 (C=O), 1587 (C=C), 1568 (C=C), 1495 (C=C); ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ_H 5.24 (2H, s, CH_2), 7.24 (1H, t, *J* = 7.0 Hz, H-4′), 7.30 (2H, t, *J* = 7.4 Hz, H-3′,5′), 7.37 (2H, d, *J* = 7.4 Hz, H-2′,6′), 7-96-8.00 (1H, m, H-8), 8.02 (1H, d, *J* = 8.0 Hz, H-5), 8.33 (1H, d, *J* = 8.0 Hz, H-4), 8.52-8.58 (2H, m, H-7, H-9); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) δ_C 43.0 (CH_2), 121.8 (quat., C-3a),

122.6 (quat., C-9a), 127.0 (CH, C-4′), 127.5 (2 × CH, C-2′, C-6′), 128.29 (2 × CH, C-3′, C-5′), 128.32 (quat., C-3b), 128.8 (C-8), 129.3 (quat., C-6), 129.8 (quat., C-6a), 131.2 (CH, C-4), 131.3 (CH, C-5), 131.8 (CH, C-7), 132.8 (CH, C-9), 137.0 (quat., C-1′), 162.86 (C=O, C-3), 162.91 (C=O, C-1); ESI-MS = 368, 366 (M + H).

2.5 General method for the preparation of 6-hydrazino-1*H*-benz[*de*]isoquinoline-1,3(2*H*)-diones 10

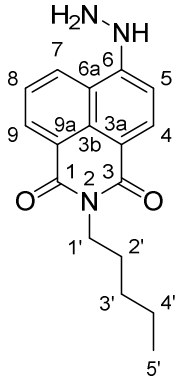
The 2-alkyl-6-bromobenz[*de*]isoquinolin-1,3(2*H*)-dione **9a-c** (1 equiv.) was reacted with hydrazine hydrate (2 equiv.) in 2-butanol (25 mL) under reflux. After 2 hours, additional hydrazine hydrate (2 equiv.) was added to the solution and after an additional 4 hours, the solution was allowed to cool to room temperature. The precipitate which formed was washed with diethyl ether to give the desired product **10a-c**.

*2.5.1 2-Propyl-6-hydrazino-1*H*-benz[*de*]isoquinoline-1,3(2*H*)-dione (10a)*



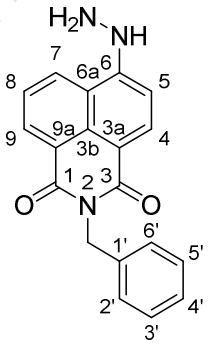
Yield of **10a**: 81%; Orange solid; mp 225-230 °C; FTIR (nujol, ν/cm^{-1}): 3375 (NH), 3316 (NH), 1668 (C=O), 1635 (C=O), 1575 (C=C), 1539 (C=C); ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ_H 0.90 (3H, t, *J* = 7.6 Hz, H-3′), 1.62 (2H, sextet, *J* = 7.6 Hz, H-2′), 3.97 (2H, t, *J* = 7.6 Hz, H-1′), 4.67 (2H, s, NH_2), 7.24 (1H, d, *J* = 8.8 Hz, H-5), 7.61-7.65 (1H, m, H-8), 8.28 (1H, d, *J* = 8.8 Hz, H-4), 8.41 (1H, d, *J* = 7.2 Hz, H-9), 8.61 (1H, d, *J* = 7.6 Hz, H-7), 9.12 (1H, broad s, NH); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ_C 12.7 (CH_3, C-3′), 23.1 (CH_2, C-2′), 42.9 (CH_2, C-1′), 109.1 (CH, C-5), 113.7 (quat., C-3a), 121.1 (quat., C-6a), 124.7 (quat., C-9a), 126.5 (CH, C-8), 129.5 (CH, C-7), 131.5 (quat., C-3b), 132.4 (CH, C-9), 135.2 (CH, C-4), 149.1 (quat., C-6), 165.2 (C=O, C-3), 165.8 (C=O, C-1); ESI-MS = 270 (M + H).

*2.5.2 2-Pentyl-6-hydrazino-1*H*-benz[*de*]isoquinoline-1,3(2*H*)-dione (10b)*



Yield of **10b**: 92%; Orange solid; mp 155-157 °C; FTIR (neat, *v*/cm^{-1}): 3283 (NH), 3265 (NH), 1695 (C=O), 1641 (C=O), 1577 (C=C), 1541 (C=C); ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ_H 0.87 (3H, t, *J* = 7.2 Hz, H-5′), 1.28-1.34 (4H, m, H-3′, H-4′), 1.60 (2H, quintet, *J* = 7.6 Hz, H-2′), 3.99 (2H, t, *J* = 7.6 Hz, H-1′), 4.67 (2H, s, NH_2), 7.24 (1H, d, *J* = 8.4 Hz, H-5), 7.61-7.65 (1H, m, H-8), 8.28 (1H, d, *J*= 8.8 Hz, H-4), 8.41 (1H, d, *J* = 7.2 Hz, H-9), 8.60 (1H, d, *J* = 8.4 Hz, H-7), 9.12 (1H, broad s, NH); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ_C 15.3 (CH_3, C-5′), 24.1 (CH_2, C-4′), 29.5 (CH_2, C-2′), 31.0 (CH_2, C-3′), 41.3 (CH_2, C-1′), 109.1 (CH, C-5), 113.7 (quat., C-3a), 121.0 (quat., C-6a), 124.6 (quat., C-9a), 126.4 (CH, C-8), 129.2 (CH, C-7), 131.4 (quat., C-3b), 132.3 (CH, C-9), 135.1 (CH, C-4), 149.0 (quat., C-6), 165.1 (C=O, C-3), 165.7 (C=O, C-1); ESI-MS = 298 (M + H).

*2.5.3 2-Benzyl-6-hydrazino-1*H*-benz[*de*]isoquinoline-1,3(2*H*)-dione (10c)*

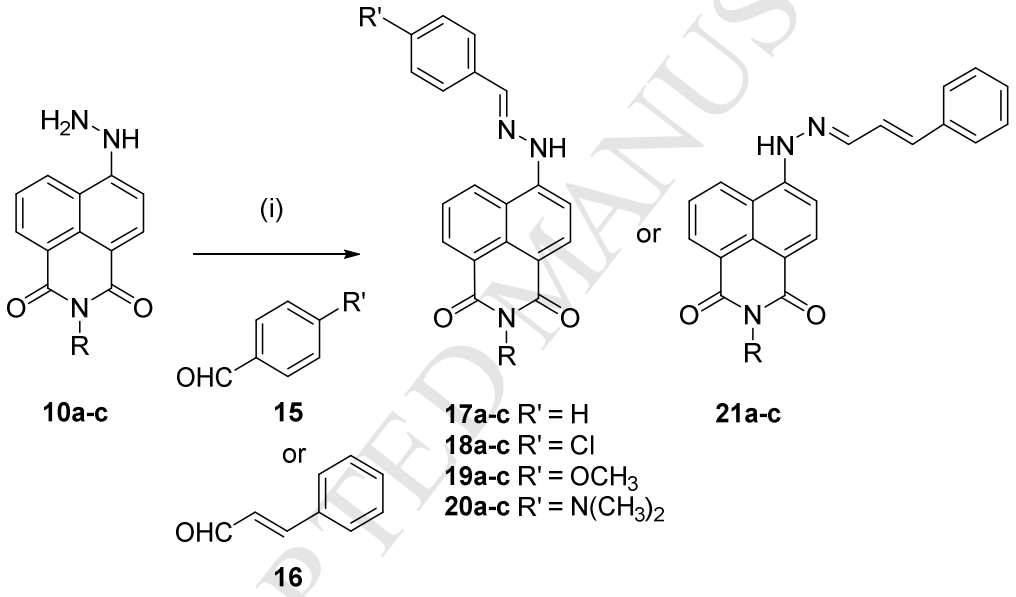


Yield of **10c**: 89%; Orange solid; mp 202-207 °C; FTIR (nujol, *v*/cm^{-1}): 3315 (NH), 3245 (NH), 1683 (C=O), 1646 (C=O), 1576 (C=C), 1539 (C=C); ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ_H 4.69 (2H, s, NH_2), 5.21 (2H, s, CH_2), 7.21-7.33 (6H, m , H-5, H-2′, H-3′, H-4′, H-5′, H-6′), 7.62-7.66 (1H, m, H-8), 8.30 (1H, d, *J*= 8.8 Hz, H-4), 8.43 (1H, d, *J* = 7.2 Hz, H-9), 8.63 (1H, d, *J* = 8.4 Hz, H-7), 9.13 (1H, broad s, NH); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ_C 44.6 (CH_2), 109.2 (CH, C-

5), 113.4 (quat., C-3a), 121.1 (quat., C-6a), 124.5 (quat., C-9a), 126.5 (CH, C-8), 128.8 (CH, C-4′), 129.6 (CH, C-7), 130.0 (2 × CH, C-2′, C-6′), 130.1 (2 × CH, C-3′, C-5′), 131.5 (quat., C-3b), 132.7 (CH, C-9), 135.5 (CH, C-4), 140.3 (quat., C-1′), 149.3 (quat., C-6), 165.2 (C=O, C-3), 165.9 (C=O, C-1); ESI-MS = 368 (M + H).

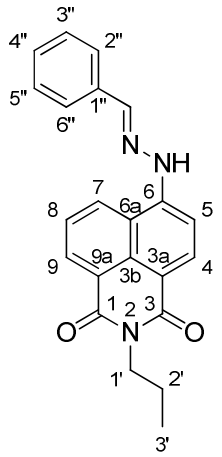
2.8 General method for the coupling of hydrazines 10 to aryl aldehydes

The hydrazines **10a-c** (1 equiv.) and arylaldehydes (1.1 equiv.) were dissolved in ethanol (25 mL) and stirred at 80 °C for 2 hours with five drops of glacial acetic acid as a catalyst. The solution was then allowed to cool and the formed precipitate was taken to obtain final compounds **17-21**.



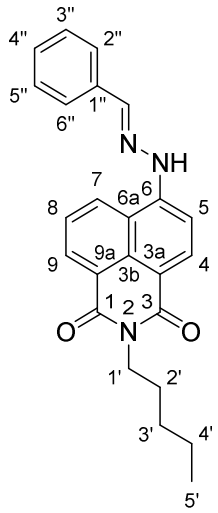
Scheme 4. Secondary reaction of substituted aryl aldehydes **15** and **16** with 4-hydrazino-1,8-naphthalimides **10a-c** to form fluorescent hydrazones **17-21**. Reagents and conditions: (i) EtOH, reflux, 2 h.

*2.8.1 6-(2-Benzylidenehydrazinyl)-2-propyl-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (15a)*



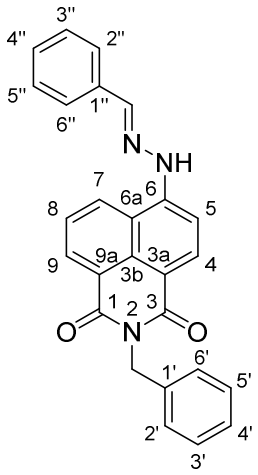
Yield of **15a**: 76%; Orange crystalline solid; mp 213 °C; FTIR (nujol, *v*/cm^{-1}): 3266 (NH), 1687 (C=O), 1635 (C=O), 1601 (C=N), 1585 (C=C), 1558 (C=C), 1539 (C=C); ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ_H 0.97 (3H, t, *J* = 7.6 Hz, CH-3′), 1.72 (2H, sextet, J=7.6 Hz, CH-2′), 4.07 (2H, t, *J* = 7.6Hz, H-1′), 7.40-7.49 (3H, m, H-3″, H-4″, H-5″), 7.72-7.76 (1H, m, H-8), 7.83 (2H, d, *J* = 7.6 Hz, H-2″, H-6″), 7.87 (1H, d, *J* = 8.4 Hz, H-5), 8.39 (1H, s, N=CH), 8.47 (1H, d, *J* = 8.4 Hz, H-4), 8.52 (1H, d, *J* = 7.2 Hz, H-9), 8.67 (1H, d, *J* = 8.4 Hz, H-7), 10.65 (1H, s, NH); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ_C 12.8 (CH_3, C-3′), 23.1 (CH_2, C-2′), 43.0 (CH_2, C-1′), 109.1 (CH, C-5), 114.3 (quat., C-3a), 120.9 (quat., C-6a), 124.9 (quat., C-9a), 126.9 (CH, C-8), 128.7 (2 × CH, C-2″, C-6″), 128.9 (CH, C-7), 130.7 (2 × CH, C-3″, C-5″), 131.5 (quat., C-3b), 131.4 (CH, C-4″), 132.5 (CH, C-9), 135.1 (CH, C-4), 136.9 (quat., C-1″), 145.5 (CH, N=CH), 148.1 (quat., C-6), 165.1 (C=O, C-3), 165.7 (C=O, C-1); ESI-MS = 358 (M + H); *Anal. Calcd. for* $4(C_{22}H_{19}N_3O_2).H_2O$ (1446): C, 73.01; H, 5.43; N, 11.61. Found: C, 73.16; H, 5.51; N, 11.44%.

*2.8.2 6-(2-Benzylidenehydrazinyl)-2-pentyl-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (15b)*



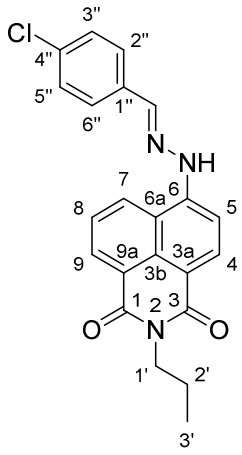
Yield of **15b**: 34%; Bright orange crystalline solid; mp 230-232 °C; FTIR (nujol, *v*/cm^{-1}): 3283 (NH), 1682 (C=O), 1639 (C=O), 1615 (C=N), 1585 (C=C), 1557 (C=C), 1506 (C=C);^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ_H 0.90-0.93 (3H, m, H-5′), 1.36-1.41 (4H, m, H-3′, H-4′), 1.71 (2H, quintet, *J* = 7.2 Hz, H-2′), 4.08-4.12 (2H, m, H-1′), 7.40-7.50 (3H, m, H-3″, H-4″, H-5″), 7.75 (1H, dd, *J* = 8.4 Hz, 7.2 Hz, H-8), 7.82-7.84 (2H, m, H-2″, H-6″), 7.87 (1H, d, *J* = 8.4 Hz, H-5), 8.39 (1H, s, N=CH), 8.47 (1H, d, *J* = 8.4 Hz, H-4), 8.53 (1H, dd, *J* = 7.2 Hz, 1.2 Hz, H-9), 8.68 (1H, dd, *J* = 8.4 Hz, 1.2 Hz, H-7), 10.65 (1H, s, NH); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ_C 15.3 (CH_3, C-5′), 24.1 (CH_2, C-4′), 29.6 (CH_2, C-2′), 31.0 (CH_2, C-3′), 41.4 (CH_2, C-1′), 109.1 (CH, C-5), 114.3 (quat., C-3a), 120.9 (quat., C-6a), 124.9 (quat., C-9a), 126.9 (CH, C-8), 128.7 (2 × CH, C-2″, C-6″), 128.9 (CH, C-7), 130.7 (2 × CH, C-3″, C-5″), 131.4 (CH, C-4″), 131.5 (quat., C-3b), 132.5 (CH, C-9), 135.1 (CH, C-4), 136.9 (quat., C-1″), 145.5 (CH, N=CH), 148.1 (quat., C-6), 165.1 (C=O, C-3), 165.7 (C=O, C-1); ESI-MS = 386 (M + H); *Anal. Calcd. for* $C_{24}H_{23}N_3O_2$ (385): C, 74.78; H, 6.01; N, 10.90. Found: C, 74.71; 6.01; N, 10.86%.

*2.8.3 6-(2-Benzylidenehydrazinyl)-2-benzyl-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (15c)*



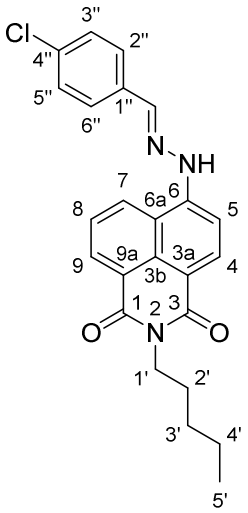
Yield of **15c**: 55%; Orange crystalline solid; mp 247-248 °C; FTIR (nujol, *v*/cm^{-1}): 3346 (NH), 1683 (C=O), 1636 (C=O), 1578 (C=N), 1539 (C=C); ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ_H 5.24 (2H, s, CH_2), 7.22 (1H, t, *J* = 7.2 Hz, H-4′), 7.28-7.35 (4H, m, H-2′, H-3′, H-5′, H-6′), 7.41-7.44 (1H, m, H-4″), 7.48 (2H, t, *J* = 7.2 Hz, H-3″, H-5″), 7.76 (1H, d, *J* = 8.4 Hz, H-5), 7.78-7.83 (3H, m, H-8, H-2″, H-6″), 8.40 (1H, d, *J* = 8.4 Hz, H-4), 8.47 (1H, s, N=CH), 8.51 (1H, d, *J* = 7.2 Hz, H-9), 8.82 (1H, d, *J* = 8.4 Hz, H-7), 11.54 (1H, s, NH); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) δ_C 42.6 (CH_2), 107.0 (CH, C-5), 110.6 (quat., C-3a), 118.7 (quat., C-6a), 121.8 (quat., C-9a), 125.0 (CH, C-8), 126.7 (2 × CH, C-2″, C-6″), 126.9 (CH, C-4′), 127.5 (2 × CH, C-2′, C-6′), 128.3 (2 × CH, C-3′, C-5′), 128.5 (CH, C-7), 128.9 (2 × CH, C-3″, C-5″), 129.3 (quat., C-3b), 129.6 (CH, C-4″), 131.1 (CH, C-9), 133.8 (CH, C-4), 134.6 (quat., C-1″), 137.7 (quat., C-1′), 144.2 (CH, N=CH), 146.8 (quat., C-6), 163.0 (C=O, C-3), 163.7 (C=O, C-1); ESI-MS = 406 (M + H); *Anal. Calcd. for* $C_{26}H_{19}N_3O_2$ (405): C, 77.02; H, 4.72; N, 10.36. Found: C, 77.00; H, 4.69; N, 10.34%.

2.8.4 6-(2-(4-Chlorobenzylidene)hydrazinyl)-2-propyl-1H-benzo[de]isoquinoline-1,3(2H)-dione (16a)



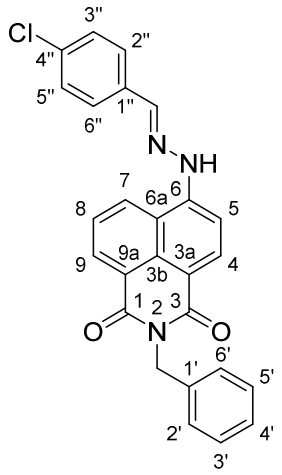
Yield of **16a**: 77%; Orange crystalline solid; mp 255-258 °C; FTIR (nujol, *ν*/cm^{-1}): 3262 (NH), 1687 (C=O), 1635 (C=O), 1616 (C=N), 1596 (C=C), 1587 (C=C); ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ_H 0.96 (3H, t, *J* = 7.6 Hz, H-3′), 1.72 (2H, sextet, *J* = 7.6 Hz, H-2′), 4.07 (2H, t, *J* = 7.6 Hz, H-1′), 7.50 (2H, H-3″, 5″), 7.76 (1H, dd, *J* = 8.4 Hz, 7.2 Hz, H-8), 7.85 (2H, H-2″, 6″), 7.88 (1H, d, *J* = 8.4 Hz, H-5), 8.38 (1H, s, N=CH), 8.48 (1H, d, *J* = 8.4 Hz, H-4), 8.53 (1H, dd, *J* = 7.2 Hz, 0.8 Hz, H-9), 8.68 (1H, dd, *J* = 8.4 Hz, 0.8 Hz, H-7), 10.73 (1H, s, NH); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ_C 12.8 (CH_3, C-3′), 23.1 (CH_2, C-2′), 43.0 (CH_2, C-1′), 109.3 (CH, C-5), 114.6 (quat., C-3a), 121.0 (quat., C-6a), 124.9 (quat., C-9a), 127.0 (CH, C-8), 128.9 (CH, C-7), 130.1 (2 × CH, C-2″, C-6″), 130.8 (2 × CH, C-3″, C-5″), 131.5 (quat., C-3b), 132.6 (CH, C-9), 135.1 (CH, C-4), 135.8 (quat., C-1″), 136.4 (quat., C-4″), 144.0 (CH, N=CH), 147.9 (quat., C-6), 165.1 (C=O, C-3), 165.7 (C=O, C-1); ESI-MS = 392 (M + H); *Anal. Calcd. for* $C_{22}H_{16}ClN_3O_2.C_2H_5OH$ (438): C, 65.83; H, 5.52; N, 9.60. Found: C, 65.92; H, 5.15; N, 9.98%.

*2.8.5 6-(2-(4-Chlorobenzylidene)hydrazinyl)-2-pentyl-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (16b)*



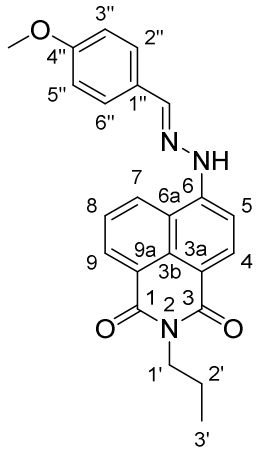
Yield of **16b**: 91%; Orange solid; mp 196-197 °C; FTIR (neat, *v*/cm^{-1}): 3248 (NH), 1680 (C=O), 1627 (C=O), 1610 (C=N), 1570 (C=C), 1560 (C=C); ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ_H 0.89-0.93 (3H, m, H-5′), 1.36-1.39 (4H, m, H-3′, H-4′), 1.69 (2H, quintet, *J* = 7.2 Hz, H-2′), 4.08 (2H, t, *J* = 7.6 Hz, H-1′), 7.70-7.74 (1H, m, H-8), 7.81-7.84 (3H, m, H-5, H-2″, H-6″), 8.34 (1H, s, N=CH), 8.44 (1H, d, *J* = 8.4 Hz, H-4), 8.50 (1H, d, *J* = 7.2 Hz, H-9), 8.63 (1H, d, *J* = 8.4 Hz, H-7), 10.68 (1H, s, NH); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ_C 15.3 (CH_3, C-5′), 24.1 (CH_2, C-4′), 29.5 (CH_2, C-2′), 31.0 (CH_2, C-3′), 41.4 (CH_2, C-1′), 109.2 (CH, C-5), 114.5 (quat., C-3a), 120.9 (quat., C-6a), 124.9 (quat., C-9a), 126.9 (CH, C-8), 128.9 (CH, C-7), 130.1 (2 × CH, C-2″, C-6″), 130.8 (2 × CH, C-3″, C-5″), 131.4 (quat., C-3b), 132.5 (CH, C-9), 135.0 (CH, C-4), 135.8 (quat., C-1″), 136.4 (quat., C-4″), 144.0 (CH, N=CH), 147.8 (quat., C-6), 165.0 (C=O, C-3), 165.6 (C=O, C-1); ESI-MS = 420 (M + H); *Anal. Calcd. For* $C_{24}H_{22}ClN_3O_2.H_2O$ (438): C, 65.83; H, 5.52; N, 9.60. Found: C, 65.85; H, 5.49; N, 9.60%.

*2.8.6 6-(2-(4-Chlorobenzylidene)hydrazinyl)-2-benzyl-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (16c)*



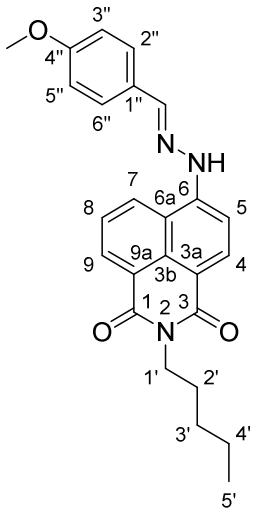
Yield of **16c**: 81%; Orange powder; mp 272-275 °C; FTIR (nujol, *v*/cm^{-1}): 3313 (NH), 1688 (C=O), 1642 (C=O), 1612 (C=N), 1584 (C=C), 1564 (C=C); ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ_H 5.23 (2H, s, CH_2), 7.23 (1H, t, *J* = 7.2 Hz, H-4′), 7.35-7.28 (4H, m, H-2′, H-3′, H-5′, H-6′), 7.53 (2H, d, *J* = 8.4 Hz, H-3″, H-5″), 7.76 (1H, d, *J* = 8.4 Hz, H-5), 7.79-7.85 (3H, H-8, H-2″, H-6″), 8.40 (1H, d, *J* = 8.4 Hz, H-4), 8.44 (1H, s, N=CH), 8.50 (1H, d, *J* = 7.2 Hz, H-9), 8.81 (1H, d, *J* = 8.4 Hz, H-7), 11.59 (1H, s, NH); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) δ_C 42.6 (CH_2), 107.1 (CH, C-5), 110.8 (quat., C-3a), 118.7 (quat., C-6a), 121.8 (quat., C-9a), 125.0 (CH, C-8), 126.9 (CH, C-4′), 127.5 (2 × CH, C-2′, C-6′), 128.3 (4 × CH, C-3′, C-5′, C-2″C, C-6″), 128.5 (CH, C-7), 128.9 (2 × CH, C-3″, C-5″), 129.2 (quat., C-3b), 131.1 (CH, C-9), 133.5 (quat., C-1″), 133.7 (CH, C-4), 133.8 (quat., C-4″), 137.7 (quat., C-1′), 142.7 (CH, N=CH), 146.6 (quat., C-6), 162.9 (C=O, C-3), 163.7 (C=O, C-1); ESI-MS = 440 (M + H); *Anal. Calcd. for* $C_{26}H_{18}ClN_3O_2$ (440): C, 70.99; H, 4.12; N, 9.55. Found: C, 71.22; H, 4.15; N,9.54%.

*2.8.7 6-(2-(4-Methoxybenzylidene)hydrazinyl)-2-propyl-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (17a)*



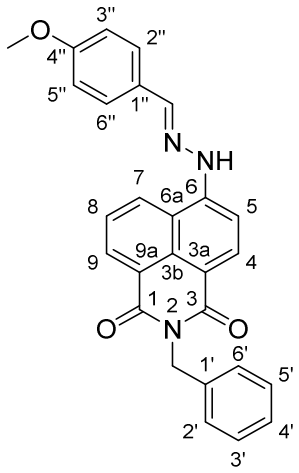
Yield of **17a**: 92%; orange-red crystalline solid; mp 226-227 °C; FTIR (nujol, *v*/cm^{-1}: 3244 (NH), 1680 (C=O), 1635 (C=O), 1602 (C=N), 1587 (C=C), 1516 (C=C); ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ_H 0.97 (3H, t, *J* = 7.6 Hz, H-3′), 1.72 (2H, sextet, *J* = 7.6 Hz, H-2′), 3.87 (3H, s, OCH_3), 4.05-4.09 (2H, m, H-1′), 7.03 (2H, H-3″, 5″), 7.73 (1H, dd, *J* = 8.4 Hz, 7.2 Hz, H-8), 7.78 (2H, H-2″, 6″), 7.84 (1H, d, *J* = 8.4 Hz, H-5), 8.34 (1H, s, N=CH), 8.46 (1H, d, *J* = 8.8 Hz, H-4), 8.52 (1H, dd, *J* = 0.8, 7.2 Hz, H-9), 8.67 (1H, dd, *J* = 0.8, 8.4 Hz, H-7), 10.54 (1H, s, NH); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ_C 12.8 (CH_3, C-3′), 23.1 (CH_2, C-2′), 42.9 (CH_2, C-1′), 56.7 (CH_3, OCH_3), 108.8 (CH, C-5), 113.8 (quat., C-3a), 116.1 (2 × CH, C-3″, C-5″), 120.9 (quat., C-6a), 124.8 (quat., C-9a), 126.7 (CH, C-8), 128.9 (CH, C-7), 129.5 (quat., C-1″), 130.2 (2 × CH, C-2", C-6″), 131.6 (quat., C-3b), 132.5 (CH, C-9), 135.2 (CH, C-4), 145.6 (CH, N=CH), 148.3 (quat., C-6), 163.0 (quat., C-4″), 165.1(C=O, C-3), 165.8 (C=O, C-1); ESI-MS = 388 (M + H); *Anal. Calcd. for* $C_{23}H_{21}N_3O_3$ (387): C, 71.30; H, 5.46; N, 10.85. Found: C, 71.12; H, 5.53; N, 10.76%.

*2.8.8 6-(2-(4-Methoxybenzylidene)hydrazinyl)-2-pentyl-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (17b)*



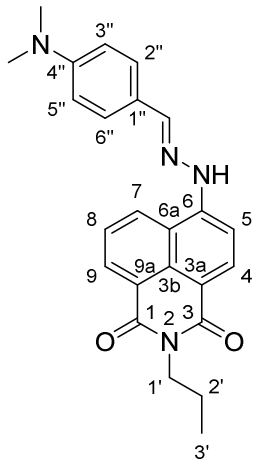
Yield of **17b**: 48%; Orange-red solid; mp 202-204 °C; FTIR (neat, ν/cm^{-1}): 3316 (NH), 1684 (C=O), 1636 (C=O), 1609 (C=N), 1570 (C=C), 1558 (C=C), 1548 (C=C), 1508 (C=C); ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ_H 0.90-0.93 (3H, m, H-5′), 1.36-1.40 (4H, m, H-3′, H-4′), 1.70 (2H, quintet, J = 7.2 Hz, H-2′), 3.87 (3H, s, OCH_3), 4.08-4.12 (2H, m, H-1′), 7.03 (2H, m, H-3″, 5″), 7.73 (1H, dd, J = 8.4 Hz, 7.2 Hz, H-8), 7.77 (2H, m′, H-2″, 6″), 7.83 (1H, d, J = 8.4 Hz, H-5), 8.34 (1H, s, N=CH), 8.46 (1H, d, J = 8.4 Hz, H-4), 8.52 (1H, dd, J = 0.8, 7.2 Hz, H-9), 8.67 (1H, dd, J = 0.8, 8.4 Hz, H-7), 10.53 (1H, s, NH); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ_C 15.3 (CH_3, C-5′), 24.1 (CH_2, C-4′), 29.6 (CH_2, C-2′), 31.0 (CH_2, C-3′), 41.4 (CH_2, C-1′), 56.7 (CH_3, OCH_3), 108.9 (CH, C-5), 113.8 (quat., C-3a), 116.1 (2 × CH, C-3″, C-5″), 120.9 (quat., C-6a), 124.8 (quat., C-9a), 126.7 (CH, C-8), 128.9 (CH, C-7), 129.5 (quat., C-1″), 130.2 (2 × CH, C-2″, C-6″), 131.5 (quat., C-3b), 132.5 (CH, C-9), 135.2 (CH, C-4), 145.6 (CH, N=CH), 148.3 (quat., C-6), 163.0 (quat., C-4″), 165.1(C=O, C-3), 165.7 (C=O, C-1); ESI-MS = 416 (M + H); *Anal. Calcd. for* $C_{25}H_{25}N_3O_3$ (415): C, 72.27; H, 6.07; N, 10.11. Found: C, 72.29; H, 6.13; N, 10.11%.

*2.8.9 6-(2-(4-Methoxybenzylidene)hydrazinyl)-2-benzyl-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (17c)*



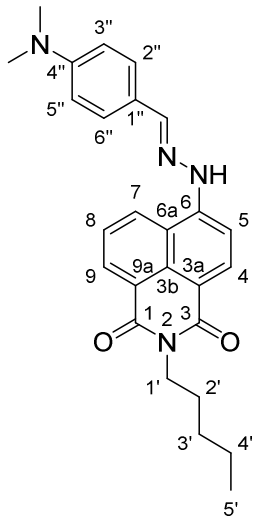
Yield **17c**: 76%; Orange-red solid; mp 226-228 °C; FTIR (neat, ν/cm^{-1}): 3319 (NH), 1684 (C=O), 1636 (C=O), 1608 (C=N), 1570 (C=C), 1558 (C=C), 1541 (C=C), 1508 (C=C); ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ_H 3.82 (3H, s, OCH_3), 5.23 (2H, s, CH_2), 7.04 (2H, d, J = 8.4 Hz, H-3″, H-5″), 7.22 (1H, t, J = 7.2 Hz, H-4′), 7.28-7.35 (4H, m, H-2′, H-3′, H-5′, H-6′), 7.72 (1H, d, J = 8.4 Hz, H-5), 7.75-7.81 (3H, m, H-8, H-2″, H-6″), 8.38 (1H, d, J = 8.4 Hz, H-4), 8.42 (1H, s, N=CH), 8.50 (1H, d, J = 7.2 Hz, H-9), 8.81 (1H, d, J = 8.4 Hz, H-7), 11.42 (1H, s, NH); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) δ_C 42.5 (CH_2), 55.3 (CH_3, OCH_3), 106.6 (CH, C-5), 110.1 (quat., C-3a), 114.3 (2 × CH, C-3″, C-5″), 118.5 (quat., C-6a), 121.8 (quat., C-9a), 124.8 (CH, C-8), 126.9 (CH, C-4′), 127.1 (CH, C-1″), 127.4 (2 × CH, C-2′, C-6′), 128.25 (2 × CH, C-3′, C-5′), 128.31 (2 × CH, C-2″, C-6″), 128.5 (CH, C-7), 129.3 (quat., C-3b), 131.0 (CH, C-9), 133.8 (CH, C-4), 137.8 (quat., C-1′), 144.3 (CH, N=CH), 146.9 (quat., C-6), 160.5 (quat., C-4″), 162.9 (C=O, C-3), 163.7 (C=O, C-1); ESI-MS = 436 (M + H); *Anal. Calcd. for* $C_{27}H_{21}N_3O_3$ (435): C, 74.47; H, 4.86; N, 9.65. Found: C, 74.22; H, 4.81; N, 9.58%.

*2.8.10 6-(2-(4-Dimethylaminobenzylidene)hydrazinyl)-2-propyl-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (18a)*



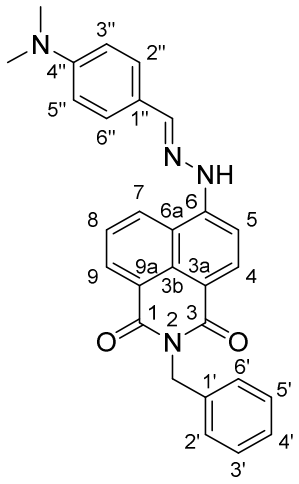
Yield of **18a**: 80%; Dark purple crystalline solid; mp 240-241°C; FTIR (nujol, *v*/cm^{-1}): 3258 (NH), 1680 (C=O), 1628 (C=O), 1611 (C=N), 1586 (C=C), 1527 (C=C); ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ_H 0.97 (3H, t, *J* = 7.6 Hz, H-3′), 1.72 (2H, sextet, *J* = 7.6 Hz, H-2′), 3.04 (6H, s, $N(CH_3)_2$), 4.05-4.09 (2H, m, H-1′), 6.81 (2H, m, *J* = 8.8 Hz, H-3″, 5″), 7.66 (2H, m, *J* = 8.8 Hz, H-2″, H-6″), 7.71 (1H, dd, *J* = 7.2, 8.4 Hz, H-8), 7.79 (1H, d, *J* = 8.4 Hz, H-5), 8.28 (1H, s, N=CH), 8.45 (1H, d, *J* = 8.4 Hz, H-4), 8.51 (1H, dd, *J* = 0.8, 7.2 Hz, H-9), 8.67 (1H, dd, *J* = 0.8, 8.4 Hz, H-7), 10.41 (1H, s, NH); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ_C 12.8 (CH_3, C-3′), 23.1 (CH_2, C-2′), 41.2 (2 × CH_3, $N(CH_3)_2$), 42.9 (CH_2, C-1′), 108.5 (CH, C-5), 112.9 (quat., C-3a), 113.8 (2 × CH, C-3″, C-5″), 120.8 (quat., C-6a), 124.4 (quat., C-1″), 124.7 (quat., C-9a), 126.5 (CH, C-8), 129.1 (CH, C-7), 130.1 (2 × CH, C-2″, C-6″), 131.6 (quat., C-3b), 132.5 (CH, C-9), 135.4 (CH, C-4), 146.9 (CH, N=CH), 148.6 (quat., C-6), 153.6 (quat., C-4″), 165.2 (C=O, C-3), 165.9 (C=O, C-1); ESI-MS = 401 (M + H); *Anal. Calcd. for* $C_{24}H_{24}N_4O_2$ (400): C, 71.98; H, 6.04; N, 13.99. Found: C, 71.87; H, 6.22; N, 13.83%.

*2.8.11 6-(2-(4-Dimethylaminobenzylidene)hydrazinyl)-2-pentyl-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (18b)*



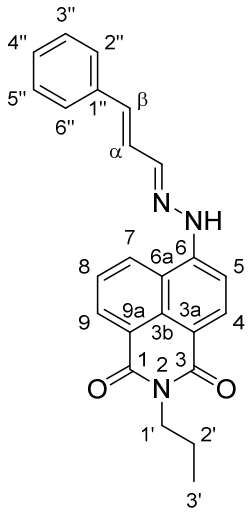
Yield of **18b**: 21%; Dark purple solid; mp 199-202 °C; FTIR (nujol, ν/cm^{-1}): 3309 (NH), 1679 (C=O), 1639 (C=O), 1613 (C=N), 1585 (C=C), 1572 (C=C), 1527 (C=C); ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ_H 0.90-0.93 (3H, m, H-5′), 1.35-1.42 (4H, m, H-3′, H-4′), 1.70 (2H, quintet, J = 7.2 Hz, H-2′), 3.03 (6H, s, $N(CH_3)_2$), 4.09-4.12 (2H, m, H-1′), 6.79 (2H, m, H-3″, 5″), 7.65 (2H, m′, H-2″, 6″), 7.70 (1H, dd, J = 7.2, 8.4 Hz, H-8), 7.78 (1H, d, J = 8.4 Hz, H-5), 8.33 (1H, s, N=CH), 8.43 (1H, d, J = 8.4 Hz, H-4), 8.50 (1H, dd, J = 0.8, 7.2 Hz, H-9), 8.74 (1H, dd, J = 0.8, 8.4 Hz, H-7), 10.68 (1H, s, NH); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) δ_C 13.8 (CH_3, C-5′), 21.8 (CH_2, C-4′), 27.2 (CH_2, C-2′), 28.7 (CH_2, C-3′), 38.9 (CH_2, C-1′), 39.8 (2 × CH_3, $N(CH_3)_2$), 106.2 (CH, C-5), 109.4 (quat., C-3a), 111.9 (2 × CH, C-3″, C-5″), 118.5 (quat., C-6a), 121.8 (quat., C-9a), 121.9 (quat., C-1″), 124.5 (CH, C-8), 128.1 (2 × CH, C-2″, C-6″), 128.2 (CH, C-7), 129.3 (quat., C-3b), 130.6 (CH, C-9), 133.6 (CH, C-4), 135.9 (quat., C-6), 145.4 (CH, N=CH), 151.2 (quat., C-4″), 162.8 (C=O, C-3), 163.6 (C=O, C-1); ESI-MS = 429 (M + H); *Anal. Calcd. for* $4(C_{26}H_{28}N_4O_2).3H_2O$: C, 70.57; H, 6.61; N, 12.78. Found: C, 70.56; H, 6.57; N, 12.48%.

*2.8.12 6-(2-(4-Dimethylaminobenzylidene)hydrazinyl)-2-propyl-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (18c)*



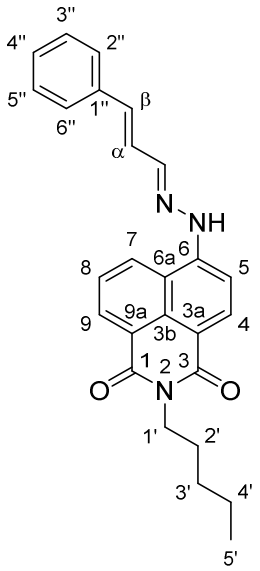
Yield of **18c**: 72%; Dark purple solid; mp 227-230 °C; FTIR (nujol, ν/cm^{-1}): 3243 (NH), 1677 (C=O), 1627 (C=O), 1604 (C=N), 1588 (C=C), 1564 (C=C), 1527 (C=C); ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ_H 2.99 (6H, s, $N(CH_3)_2$), 5.24 (2H, s, CH_2), 6.79 (2H, m, H-3″, 5″), 7.20-7.25 (1H, m, H-4′), 7.28-7.36 (4H, m, H-2′, H-3′, H-5′, H-6′), 7.64 (2H, m, H-2″, 6″), 7.68 (1H, d, J = 8.8 Hz, H-5), 7.78 (1H, dd, J = 7.6, 8.4 Hz, H-8), 8.37-8.39 (2H, m, H-4, N=CH), 8.50 (1H, dd, J = 7.6 Hz, 0.8 Hz, H-9), 8.82 (1H, dd, J = 8.4 Hz, 0.8 Hz, H-7), 11.31 (1H, s, NH); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) δ_C 39.9 (2 × CH_3, $N(CH_3)_2$), 42.7 (CH_2), 106.5 (CH, C-5), 109.6 (quat., C-3a), 112.0 (2 × CH, C-3″, C-5″), 118.6 (quat., C-6a), 121.8 (quat., C-9a), 121.9 (quat., C-1″), 124.9 (CH, C-8), 127.1 (CH, C-4′), 127.6 (2 × CH, C-2′, C-6′), 128.3 (2 × CH, C-2″, C-6″), 128.5 (2 × CH, C-3′, C-5′), 128.6 (CH, C-7), 129.5 (quat., C-3b), 131.2 (CH, C-9), 134.1 (CH, C-4), 137.9 (quat., C-1′), 145.7 (CH, N=CH), 147.1 (quat., C-6), 151.4 (quat., C-4″), 163.1 (C=O, C-3), 163.9 (C=O, C-1); ESI-MS = 449 (M + H); *Anal. Calcd. for* $C_{28}H_{24}N_4O_2 \cdot H_2O$ (466): C, 72.09; H, 5.62; N, 12.01. Found: C, 71.89; H, 5.52; N, 12.02%.

*2.8.13 6-(2-Cinnamylidenehydrazinyl)-2-propyl-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (19a)*



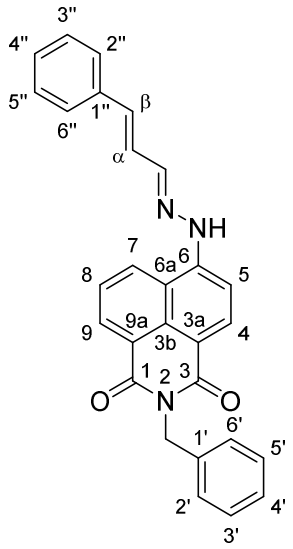
Yield of **19a**: 76%; Orange solid; mp 196-199 °C; FTIR (nujol, *ν*/cm^{-1}): 3244 (NH), 1689 (C=O), 1639 (C=O), 1621 (C=N), 1591 (C=C), 1506 (C=C); ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ_H 0.97 (3H, t, *J* = 7.6 Hz, H-3′), 1.72 (2H, sextet, *J* = 7.6 Hz, H-2′), 4.05-4.09 (2H, m, H-1′), 7.00 (1H, d, *J* = 16.0 Hz, alkene β CH), 7.16 (1H, dd, *J* = 9.2, 16.0 Hz, alkene α CH), 7.31-7.35 (1H, m, H-4″), 7.39-7.43 (2H, m, H-3″, H-5″), 7.61-7.63 (2H, m, H-2″, H-6″), 7.71-7.75 (2H, m, H-5, H-8), 8.23 (1H, d, *J* = 9.2 Hz, N=CH), 8.45 (1H, d, *J* = 8.4 Hz, H-4), 8.52 (1H, dd, *J* = 0.8, 7.2 Hz, H-9), 8.65 (1H, dd, *J* = 0.8, 8.4 Hz, H-7), 10.58 (1H, s, NH); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ_C 12.8 (CH_3, C-3′), 23.1 (CH_2, C-2′), 42.9 (CH_2, C-1′), 109.0 (CH, C-5), 114.2 (quat., C-3a), 121.0 (quat., C-6a), 124.8 (quat., C-9a), 126.8 (CH, C-8), 127.5 (CH, alkene α CH), 128.8 (2 × CH, C-2″, C-6″), 128.9 (CH, C-7), 130.4 (CH, C-4″), 130.7 (2 × CH, C-3″, C-5″), 131.5 (quat., C-3b), 132.5 (CH, C-9), 135.1 (CH, C-4), 138.4 (quat., C-1″), 139.2 (CH, alkene β CH), 147.8 (quat., C-6), 147.9 (CH, N=CH), 165.1 (C=O, C-3), 165.7 (C=O, C-1); ESI-MS = 384 (M + H); *Anal. Calcd. for* $C_{24}H_{21}N_3O_2.C_2H_5OH$ (429): C, 72.71; H, 6.34; N, 9.78. Found: C, 72.76; H, 6.41; N, 9.89%.

*2.8.14 6-(2-Cinnamylidenehydrazinyl)-2-pentyl-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (19b)*



Yield of **19b**: 82%; Orange-red crystalline solid; mp 182-183 °C; FTIR (neat, *v*/cm^{-1}): 3269 (NH), 1684 (C=O), 1632 (C=O), 1614 (C=N), 1570 (C=C), 1560 (C=C); ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ_H 0.89-0.93 (3H, m, H-5′), 1.36-1.40 (4H, m, H-3′, H-4′), 1.66-1.74 (2H, m, H-2′), 4.08-4.12 (2H, m, H-1′), 6.99 (1H, d, *J* = 12.0 Hz, alkene β CH), 7.16 (1H, dd, *J* = 9.2, 16.0 Hz, alkene α CH), 7.31-7.35 (1H, m, H-4″), 7.39-7.43 (2H, m, H-3″, H-5″), 7.61-7.63 (2H, m, H-2″, H-6″), 7.70-7.75 (2H, m, H-5, H-8), 8.22 (1H, d, *J* = 9.2 Hz, N=CH), 8.44 (1H, d, *J* = 8.4 Hz, H-4), 8.51 (1H, dd, *J* = 0.8, 7.2 Hz, H-9), 8.64 (1H, dd, *J* = 0.8, 8.4 Hz, H-7), 10.56 (1H, s, NH); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ_C 15.3 (C-5′), 24.1 (C-4′), 29.5 (C-2′), 31.0 (C-3′), 41.4 (C-1′), 109.0 (C-5), 114.3 (quat., C-3a), 121.0 (quat., C-6a), 124.8 (quat., C-9a), 126.8 (C-8), 127.5 (alkene α CH), 128.8 (C-2″, C-6″), 128.9 (C-7), 130.4 (C-4″), 130.7 (C-3″, C-5″), 131.5 (quat., C-3b), 132.5 (C-9), 135.1 (C-4), 138.4 (quat., C-1″), 139.2 (alkene β CH), 147.8 (quat., C-6), 147.9 (N=CH), 165.1 (C-3), 165.7 (C-1); ESI-MS = 412 (M + H); *Anal. Calcd. for* $C_{26}H_{25}N_3O_2.C_2H_5OH$ (458): C, 73.50; H, 6.83; N, 9.18. Found: C, 73.27; H, 6.90; N, 9.36%.

*2.8.15 6-(2-Cinnamylidenehydrazinyl)-2-benzyl-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (19c)*



Yield of **19c**: 58%; Orange solid; mp 232-234 C; FTIR (neat, *v*/cm^{-1}): 3312 (NH), 1695 (C=O), 1684 (C=O), 1636 (C=N), 1571 (C=C), 1558 (C=C), 1541 (C=C), 1508 (C=C); ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ_H 5.24 (2H, s, CH_2), 7.08 (1H, d, *J* = 16.0 Hz, alkene β CH), 7.15 (1H, dd, *J* = 8.8, 16.0 Hz, alkene α CH), 7.23 (1H, t, *J* = 7.2 Hz, H-4′), 7.28-7.35 (5H, m, H-2′, H-3′, H-5′, H-6′, H-4″), 7.41 (2H, t, *J* = 7.6 Hz, H-3″, H-5″), 7.62 (1H, d, *J* = 8.8 Hz, H-5), 7.65 (2H, d, *J* = 7.6 Hz, H-2″, H-6″), 7.79-7.83 (1H, m, H-8), 8.31 (1H, d, *J* = 8.8 Hz, N=CH), 8.40 (1H, d, *J* = 8.4 Hz, H-4), 8.51 (1H, d, *J* = 7.6 Hz, H-9), 8.82 (1H, d, *J* = 8.4 Hz, H-7), 11.45 (1H, s, NH); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) δ_C 42.5 (CH_2), 106.8 (CH, C-5), 110.5 (quat., C-3a), 118.7 (quat., C-6a), 121.8 (quat., C-9a), 125.0 (CH, C-8), 125.5 (CH, alkene α CH), 126.88 (CH, C-4′), 126.93 (2 × CH, C-2″, C-6″), 127.4 (2 × CH, C-2′, C-6′), 128.3 (2 × CH, C-3′, C-5′), 128.5 (CH, C-7), 128.6 (CH, C-4″), 128.8 (2 × CH, C-3″, C-5″), 129.3 (quat., C-3b), 131.1 (CH, C-9), 133.7 (CH, C-4), 136.1 (quat., C-1″), 137.6 (CH, alkene β CH), 137.7 (quat., C-1′), 146.4 (quat., C-6), 146.8 (CH, N=CH), 162.9 (C=O, C-3), 163.7 (C=O, C-1); ESI-MS = 432 (M + H); *Anal. Calcd. for* $C_{28}H_{21}N_3O_2$ (431): C, 77.94; H, 4.91; N, 9.74. Found: C, 77.87; H, 4.83; N, 9.69%.

3 Fluorescence excitation and emission spectra

3.1 Fluorescence properties of ANS derivatives

Fluorescent properties of ANS derivatives are given in Figure S1.

3.2 Fluorescence properties of 6-HBI derivatives

Fluorescent properties of 6-HBI derivatives are given in Figure S2, S3, S4.

4 Biological evaluation

Interpretation of multipoint inoculation plates shown in Table S1.

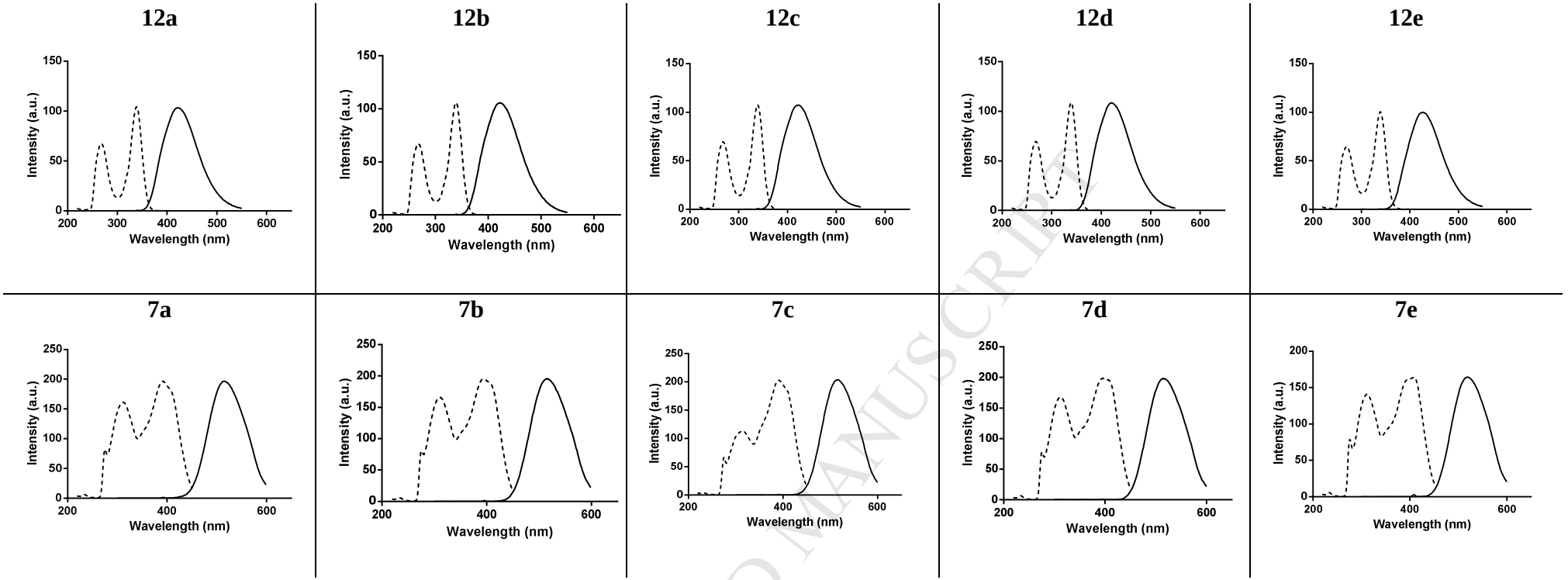


Figure S1. Emission (solid line) and excitation (dashed line) spectra for substrate **12a-e** and fluorophore **7a-e** (5×10^{-4} M in EtOH) (intensities are shown in arbitrary units).

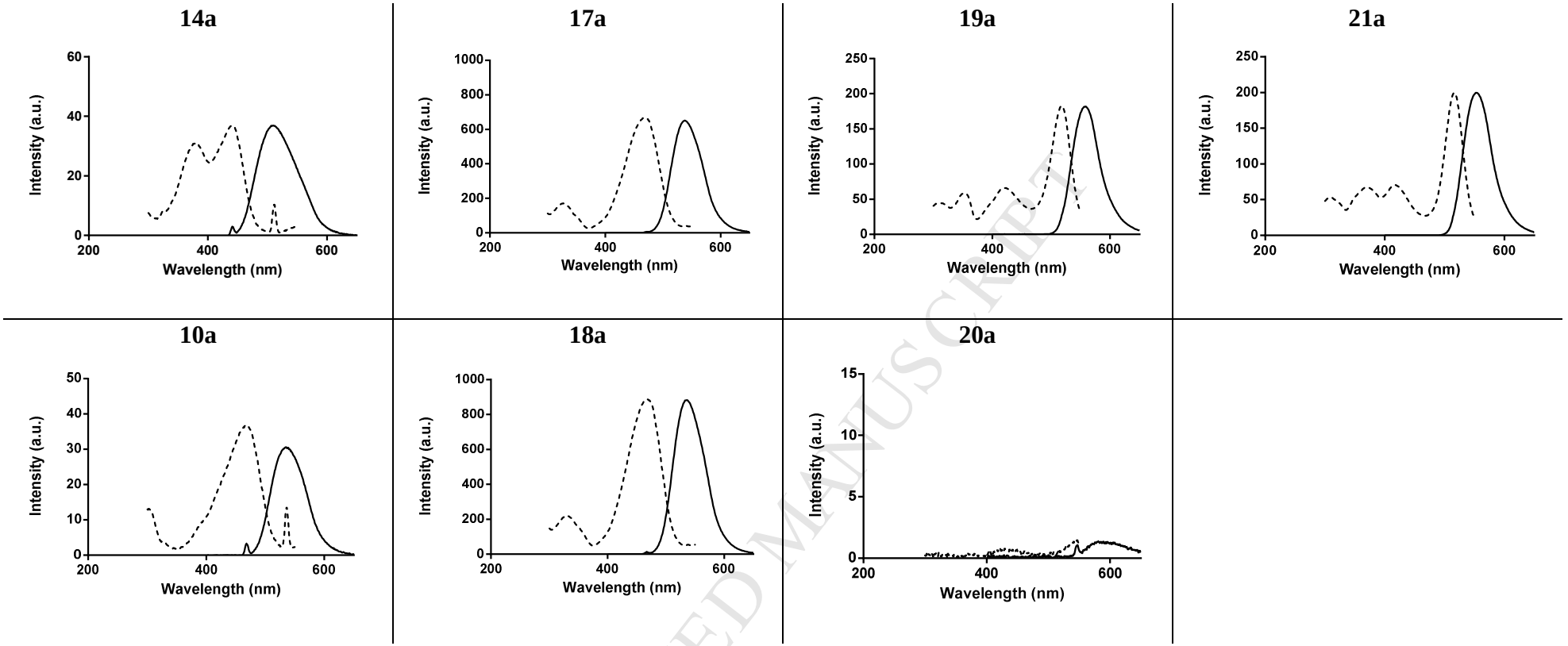


Figure S2. Emission (solid line) and excitation (dashed line) spectra for substrate **14a** fluorophore **10a** and hydrazone derivatives **19a-21a** (1×10^{-4} M in EtOH) (intensities are shown in arbitrary units).

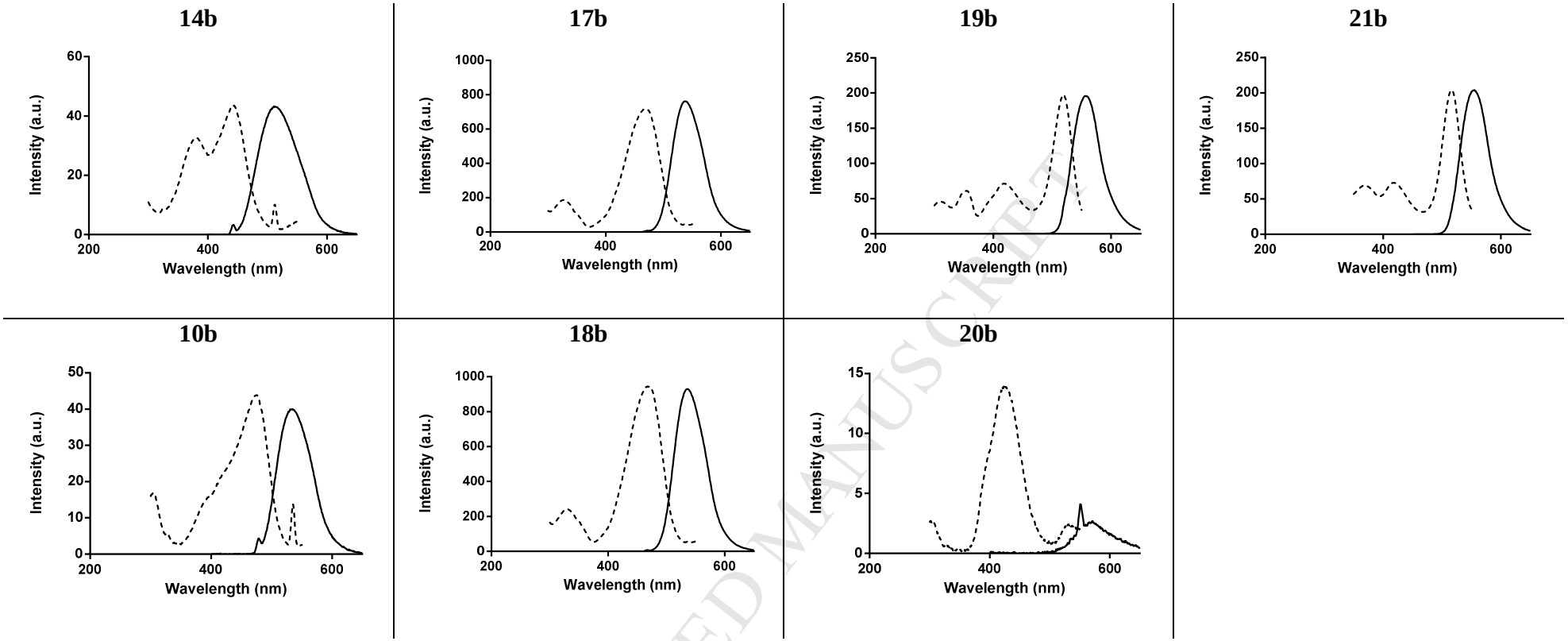


Figure S3. Emission (solid line) and excitation (dashed line) spectra for substrate **14b** fluorophore **10b** and hydrazone derivatives **19b-21b** (1×10^{-4} M in EtOH) (intensities are shown in arbitrary units).

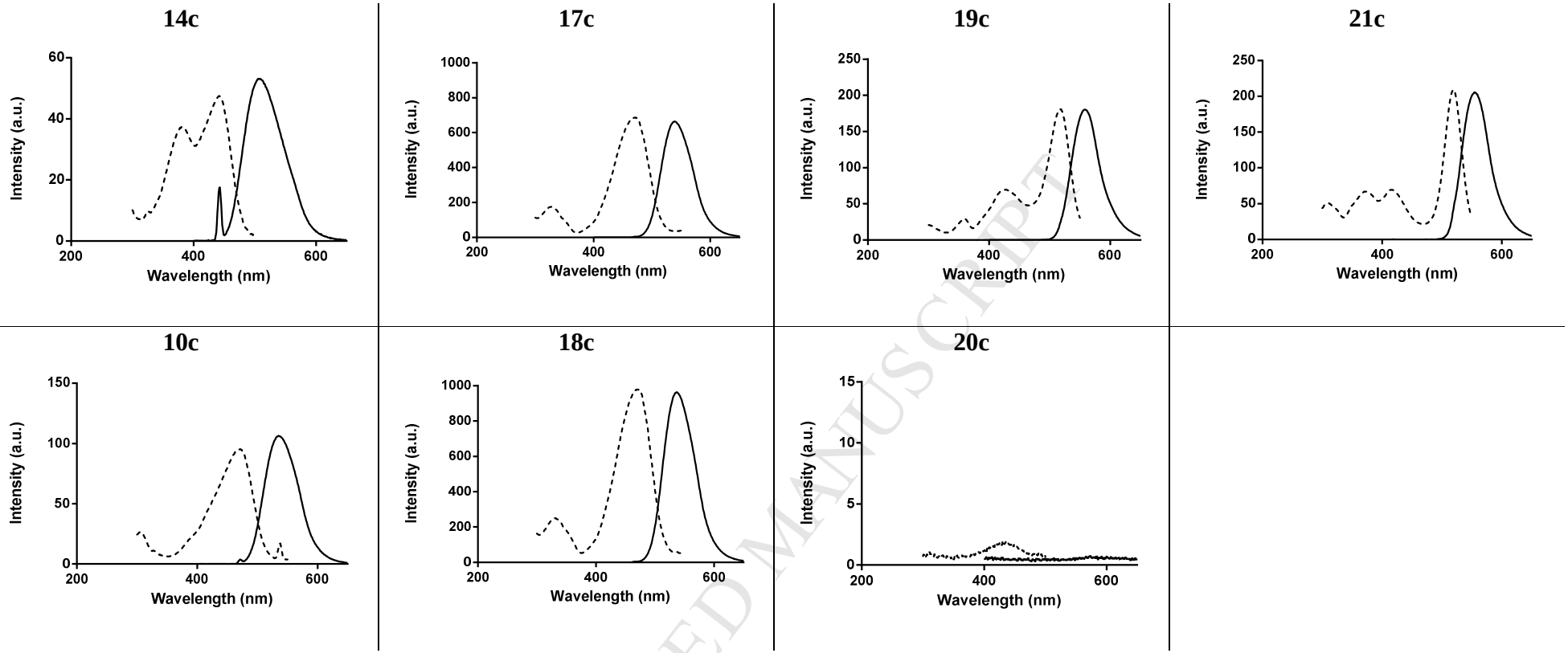


Figure S4. Emission (solid line) and excitation (dashed line) spectra for substrate **14c** fluorophore **10c** and hydrazone derivatives **19c-21c** (1×10^{-4} M in EtOH) (intensities are shown in arbitrary units).

Table S1. Inhibitory effects and fluorescence of substrates **12a-e and 14a-c** on Columbia agar against a range of bacteria and fungi (G: growth, F: fluorescence, B: blue, TrB: traces of blue, Y: yellow, ++ good growth, +/- moderate growth, - no growth.

Code	Organism	Control		12a & 12b		12c		12d		12e		14a		14b		14c	
		G	F	G	F	G	F	G	F	G	F	G	F	G	F	G	F
1	*Escherichia coli*	++	-	++	-	++	+/- B	-	-	++	TrB	++	+ Y	-	-	-	-
2	*Klebsiella pneumoniae*	++	-	++	-	++	-	-	-	++	-	++	+ Y	-	-	-	-
4	*Enterobacter cloacae*	++	-	++	-	++	+/- B	-	-	++	TrB	++	+ Y	+	+ Y	+	+ Y
5	*Serratia marcescens*	++	-	++	-	++	-	++	+/- B	++	-	++	++ Y	+	+ Y	+	+ Y
6	*Salmonella typhimurium*	++	-	++	-	++	-	-	-	++	-	++	+ Y	-	-	-	-
7	*Pseudomonas aeruginosa*	++	-	++	-	++	-	-	-	++	-	++	+ Y	-	-	-	-
8	*Yersinia enterocolitica*	++	-	++	-	++	-	-	-	++	-	++	+ Y	-	-	+	-
9	*Burkholderia cepacia*	+/-	-	+	-	+	-	+	-	+	-	+	+/- Y	+	+/- Y	+	-
10	*Acinetobacter baumannii*	++	-	++	-	++	-	-	-	++	-	++	++ Y	-	-	+	+ Y
11	*Streptococcus pyogenes*	+	-	+	-	+	-	-	-	+	-	+/-	-	-	-	-	-
12	*Staphylococcus aureus* (MRSA)	+	-	+	-	+	-	-	-	+	-	+	+ Y	-	-	-	-
13	*Staphylococcus aureus*	+	-	+	-	+	-	-	-	+	-	+	+ Y	-	-	-	-
14	*Staphylococcus epidermidis*	+	-	+	-	+	-	-	-	+	-	+	+/- Y	-	-	-	-
15	*Listeria monocytogenes*	+	-	+	-	+	-	-	-	+	-	+	+/- Y	-	-	-	-
16	*Enterococcus faecium*	+	-	+	-	+	-	-	-	+	-	+	+/- Y	-	-	-	-
17	*Enterococcus faecalis*	+	-	+	-	+	-	-	-	+	-	+	+/- Y	-	-	-	-
18	*Bacillus subtilis*	+	-	+	-	+	-	-	-	+	-	+	-	-	-	-	-
19	*Candida albicans*	+	-	+	-	+	-	-	-	+	-	+	-	-	-	-	-
20	*Candida glabrata*	+/-	-	+/-	-	+/-	-	-	-	+/-	-	-	-	-	-	-	-