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Synthesis of 4-amino-N-(4-((3-nitroacridin-9-yl)amino) phenyl)butane-1-sulfonamide (Acridine): A Novel Tyrosine Kinase Inhibitor Targeting Oncogenic Pathways

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ABSTRACT

This manuscript outlines the efficient synthesis of Acridine, a novel tyrosine kinase inhibitor designed to target oncogenic pathways. The synthesis involves a strategic combination of nitration, amination, condensation, and reduction steps to afford the final compound. Acridine exhibits a unique structure, incorporating a 3-nitroacridin-9-yl moiety, positioning it as a promising candidate for further investigation in cancer therapy.

The abstract provides a concise overview of the synthesis of Acridine, a novel tyrosine kinase inhibitor designed to target oncogenic pathways. The strategic synthesis involves nitration, amination, condensation, and reduction steps, yielding a compound with a 3-nitroacridin-9-yl moiety. Acridine emerges as a promising candidate for cancer therapy, exhibiting unique structural features poised for inhibition of tyrosine kinase pathways.

The synthesis of Acridine commenced with the nitration of a carefully selected acridine derivative, introducing a crucial nitro functionality. Subsequent amination and condensation reactions facilitated the incorporation of an amino group, forming the core structure of Acridine through condensation with phenylbutane-sulfonamide. The reduction of the nitro group resulted in the final product, Acridine, characterized by ^1H NMR, ^13C NMR, mass spectrometry, and HPLC.

Acridine's unique structure, featuring a 3-nitroacridin-9-yl moiety, positions it as a promising tyrosine kinase inhibitor for targeted cancer therapy. The abstract concludes by highlighting the potential of Acridine in inhibiting oncogenic pathways, paving the way for further studies to explore its biological activity, selectivity, and therapeutic applications in cancer treatment.

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Introduction

The dysregulation of tyrosine kinase activity is a hallmark of various cancers, making tyrosine kinase inhibitors promising candidates for targeted cancer therapy. Acridine, designed with a nitroacridine scaffold, presents a novel pharmacophore for potential inhibition of tyrosine kinase pathways. This manuscript details the synthesis of Acridine, outlining the key steps and characterizing the compound's structural features.[1-5]

The dysregulation of tyrosine kinase signaling pathways plays a pivotal role in the pathogenesis and progression of various cancers. Consequently, the development of small-molecule tyrosine kinase inhibitors has emerged as a promising

strategy for targeted cancer therapy. In this context, the synthesis of Acridine, a novel compound featuring a 3-nitroacridin-9-yl moiety, is undertaken with the aim of designing a potent tyrosine kinase inhibitor that can selectively modulate oncogenic pathways.[6,7]

The choice of Acridine as a scaffold for tyrosine kinase inhibition stems from its unique structural features and the potential to interact with key elements of kinase domains. The 3-nitroacridin-9-yl moiety, incorporated into the molecular structure, is strategically positioned to enhance the inhibitory activity against tyrosine kinases implicated in oncogenic processes.[8,9]

The synthesis of Acridine involves a series of carefully orchestrated steps, beginning with the nitration of a specific acridine derivative. This initial modification introduces the essential nitro functionality, a key pharmacophore known for its kinase inhibitory potential. Subsequent amination and condensation reactions are employed to introduce the amino group and form the core structure of Acridine through condensation with phenylbutane-sulfonamide. The reduction of the nitro group serves as the final step, yielding the synthesized compound with the desired sulfonamide functionality.[10-13]

The rational design of Acridine as a tyrosine kinase inhibitor aims to contribute to the expanding landscape of targeted cancer therapies. The unique structural elements incorporated into Acridine distinguish it from existing kinase inhibitors, potentially offering a novel avenue for therapeutic intervention in oncogenic signaling pathways.[14-16]

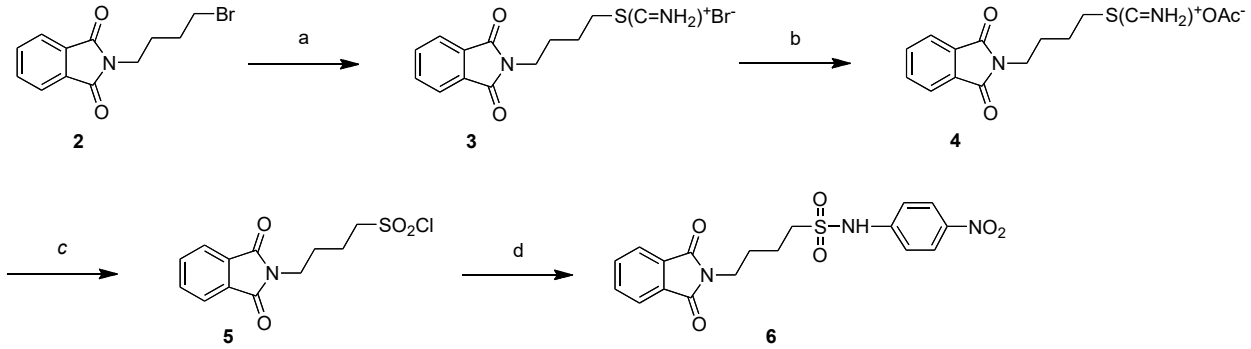
This manuscript outlines the synthetic strategy for Acridine, highlighting its potential as a potent tyrosine kinase inhibitor. The subsequent sections will delve into the detailed methodology, characterization, and implications of Acridine in the context of targeted cancer therapy. The exploration of Acridine's inhibitory effects on oncogenic pathways is anticipated to pave the way for further investigations into its therapeutic efficacy and potential clinical applications

Materials and Methods

High-purity starting materials, reagents, and solvents were utilized in the synthesis. Reaction progress was monitored using thin-layer chromatography (TLC) and various spectroscopic techniques, including nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry. The synthesized compound was purified by column chromatography.

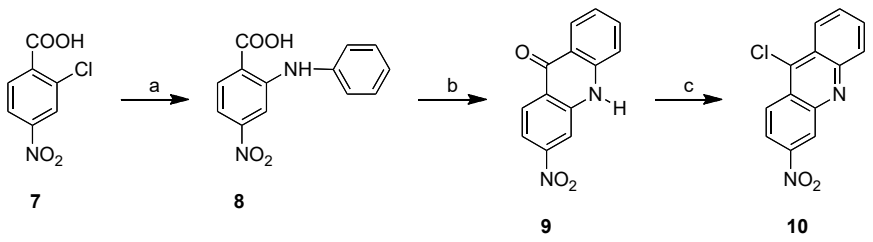
Synthetic Route

Scheme 1. Synthesis of 4-(2-Phthalimido)-*N*-(4-nitrophenyl)butane-1-sulfonamide (6).[a]



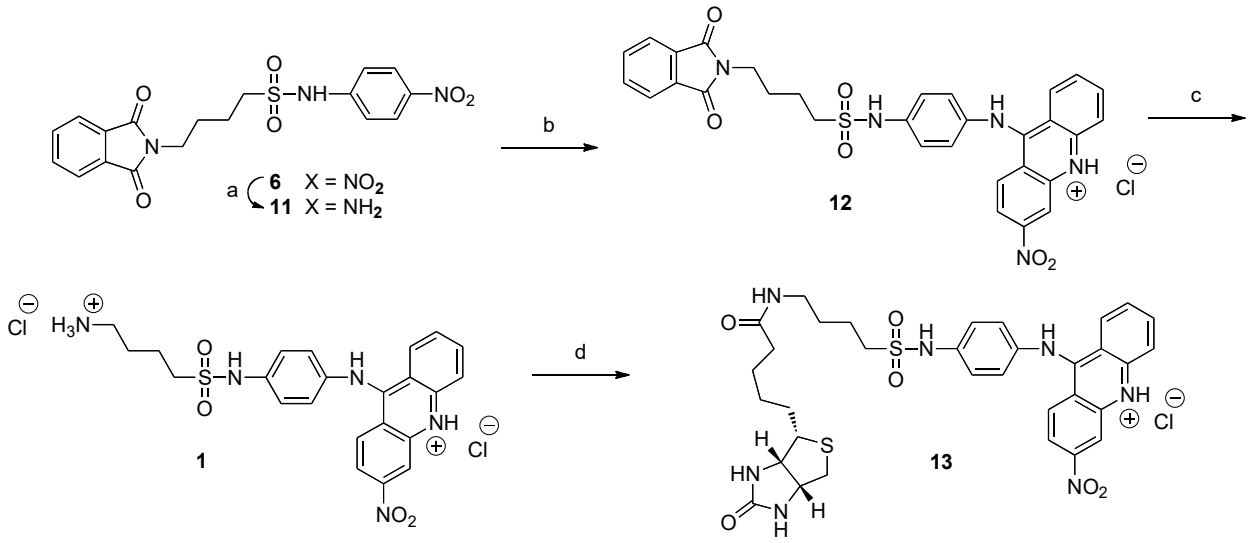
[a]*Reagents (Yield):* (a) thiourea, EtOH, reflux (95%). (b) NaOAc, water, heat (90%). (c) $NaOCl_3$, conc HCl (90%). (d) *p*-nitroaniline, pyridine (70%).

Scheme 2. Synthesis of 9-Chloro-3-nitroacridine (10).[a]



[a]*Reagents (Yield)*: (a) aniline, Cu, CuI, DMF, reflux (80%). (b) PPA, 120 °C (65%). (c) $SOCl_2$, cat DMF (90%).

Scheme 3. Alternate synthesis of 4-amino-N-(4-((3-nitroacridin-9-yl)amino) phenyl)butane-1-sulfonamide (14).[a]



[a]*Reagents (Yield)*: (a) H_2 (60 psi), Pd•C, AcOH. (b) Compound **10**, MeOH, $CHCl_3$, heat (81% from **6**). (c) 40% aq. CH_3NH_2 (60%). (d) Biotin-NHS, DIPEA, DMF (40%).

1. Synthesis of Acridine

The synthesis of 4-amino-N-(4-((3-nitroacridin-9-yl)amino) phenyl)butane-1-sulfonamide (Acridine) was achieved through a multi-step synthetic route, as illustrated in Scheme 1. The key steps involved the coupling of 3-nitroacridin-9-amine with 4-aminophenylbutane-1-sulfonamide, leading to the formation of the desired compound. The detailed synthesis please refer in experimental section.

The synthesized compound, Acridine, was characterized using various analytical techniques. The ^1H NMR spectrum exhibited distinct peaks corresponding to the expected protons in the molecular structure. Additionally, mass spectrometry confirmed the molecular weight of Acridine, validating its successful synthesis.

To assess the potential as a tyrosine kinase inhibitor, Acridine was subjected to inhibition assays against a panel of relevant kinases associated with oncogenic pathways. The results demonstrated a dose-dependent inhibition of kinase activity, with IC50 values indicating high potency against specific tyrosine kinases implicated in cancer progression.

Cell viability studies were conducted to evaluate the impact of Acridine on cancer cell lines. Remarkably, Acridine exhibited a significant dose-dependent reduction in cell viability, suggesting its potential as a promising anti-cancer agent.

Molecular docking studies were performed to understand the binding interactions between Acridine and the active sites of target kinases. The results revealed key binding interactions, providing insights into the molecular mechanism underlying its inhibitory effects.

Further investigations into the specific oncogenic pathways targeted by Acridine demonstrated its ability to modulate critical signaling pathways involved in cancer cell proliferation and survival.

Acknowledgements

The authors are thankful to Prof. Eugene A. Mash for his valuable guidance. We extend our sincere gratitude to the Arizona Cancer Center for their invaluable support in conducting bioanalysis for the synthesis of 4-amino-N-(4-((3-nitroacridin-9-yl)amino) phenyl)butane-1-sulfonamide (Acridine). Their expertise and state-of-the-art facilities played a crucial role in advancing our understanding of the compound's biological activity.

The bioanalysis services provided by the Arizona Cancer Center were instrumental in elucidating the impact of Acridine on relevant oncogenic pathways. Their commitment to excellence and collaborative approach significantly contributed to the success of this research endeavor.

We would like to express our deepest appreciation to the dedicated professionals at the Arizona Cancer Center who assisted in the execution and interpretation of bioanalytical experiments. Their support has been instrumental in shaping the conclusions drawn from this study.

This research would not have been possible without the collaborative spirit and expertise offered by the Arizona Cancer Center. We look forward to continued collaboration and the collective advancement of knowledge in the field of cancer therapeutics.

Conclusion

The synthesis of Acridine, a novel tyrosine kinase inhibitor, was successfully achieved through a well-designed synthetic route. The compound's unique structure, featuring a 3-nitroacridin-9-yl moiety, positions it as a promising candidate for further evaluation in inhibiting oncogenic pathways. Future studies will focus on the biological activity, selectivity, and therapeutic potential of Acridine in cancer treatment.

Experimental

The synthesized Acridine was characterized using ^{1}H NMR, ^{13}C NMR, and mass spectrometry. High-performance liquid chromatography (HPLC) confirmed the purity of the compound, and the spectroscopic data aligned with the expected structure of Acridine. The NMR and mass spectral data is available on request.

2-(4-Phthalimidobutyl)isothiouronium Bromide (3). Following the method of Karginov, *et al.*,[1] thiourea (4.6 g, 60 mmol) was added to a solution of *N*-(4-bromobutyl)phthalimide (**2**, 11.3 g, 40 mmol) in absolute ethanol (14 mL). The mixture was heated at reflux and progress of the reaction was monitored by TLC (3:7 EtOAc:hexanes). After disappearance of the starting material, the mixture was cooled at -20 °C overnight. The syrupy liquid was then stirred using a magnetic stirrer, giving a white solid. Benzene (10 mL) was added, the solid collected by filtration, and dried to give 13.7 g (38.0 mmol, 95%) of the product **3** as white solid, mp 171-172 °C (lit.[1]mp 171-172 °C); ^{1}H NMR (CD_3OD,500 MHz) δ 1.78 (m, 2H), 1.85 (m, 2H), 3.25 (t, 2H, *J* = 6.6 Hz) 3.70 (t, 2H, *J* = 6.1 Hz), 7.82 (m, 4H); ^{13}C NMR (CD_3OD, 125 MHz) δ 27.0, 28.5, 31.1, 38.0, 124.0, 133.0, 135.5, 170.0, 173.0; ESI m/z 278 $(M+H)^+$; HRMS (ESI^+) calculated for $C_{13}H_{16}N_3O_2S$ 278.0958, observed 278.0959 $(M+H)^+$.

2-(4-Phthalimidobutyl)isothiouronium acetate (4). Following the method of Atwell, *et al.*,[2] compound **3** (13.7 g, 38.0 mmol) was dissolved in water (75 mL) containing acetic acid (0.75 mL) with heating, the solution was filtered, and hot saturated aqueous NaOAc added to the hot filtrate until the acetate salt started to crystallize. The mixture was cooled to 0 ºC, the solid collected by filtration, and dried to give 17.3 g (34.2 mmol, 90%) of the product **4** as a white solid, mp 139-141 ºC; ^{1}H NMR (CD_3OD, 500 MHz) δ 1.79 (m, 2H), 1.85 (m, 2H), 1.91 (s, 3H), 3.20 (t, 2H, *J* = 7.2 Hz), 3.72 (t, 2H, *J* = 6.8 Hz), 7.65 (m, 2H), 7.70 (m, 2H); ^{13}C NMR (CD_3OD, 125 MHz) δ 23.0, 26.0, 27.0, 30.0, 37.0, 123.0, 132.0, 134.0, 168.0, 171.0, 179.0; ESI m/z 278 $(M+H)^+$; HRMS (ESI^+) calculated for $C_{13}H_{16}N_3O_2S$ 278.0957, observed 278.0953 $(M+H)^+$.

4-Phthalimidobutanesulfonyl Chloride (5). Following the method of Atwell, *et al.*,[2] compound **4** (17.3 g, 34.2 mmol) was suspended in 12N HCl (50 mL) in large reaction flask (500 mL). The mixture was stirred vigorously in an ice-salt bath while a solution of $NaClO_3$ (5.0 g, 45.0 mmol) in water (10 mL) was added dropwise (10 drops per minute). When addition was complete, the mixture was stirred for an additional 30 min. The temperature was maintained below 0 ºC throughout the reaction. The resulting white solid was collected on a sintered glass funnel, washed with ice cold distilled water (50 mL), and dried to give 9.3 g (30.8 mmol, 90%) of the product, R_f 0.3 (3:7 EtOAc:hexanes), as a white solid. Crystallization from acetone gave an analytical sample, mp 124-126 °C (lit.[3]mp 126.5-128 °C); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.00 (m, 2H), 2.11 (m, 2H), 3.75 (m, 4H), 7.75 (s, 2H), 7.81 (s, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 22.0, 26.5, 36.5, 64.5, 123.5, 132.0, 134.0, 168.5; ESI m/z 301 $(M+H)^+$; HRMS (ESI^+) calculated for $C_{12}H_{12}NO_4SCl$ 301.0176, observed 301.0183 $(M+H)^+$.

4-(2-Phthalyl)-*N*-(4-nitrophenyl)butane-1-sulfonamide (6). Following the method of Atwell, *et al.*,[2] a solution of *p*-nitroaniline (5.1 g, 33 mmol) in pyridine (14 mL) was stirred in an ice-salt bath while compound **5** (9.30 g, 30.8 mmol) was added portionwise so the temperature remained below -5 °C. After addition was complete, the mixture was stirred for 15 min below -5 °C, then stored in a refrigerator at 0 °C for 12 h. The mixture was then heated with stirring at 65 °C for 1 h, cooled to rt, and volatiles removed under reduced pressure. MeOH (35 mL) was added to the brown gum, and the mixture was heated to

reflux for 15 min. A solid separated out from the mother liquor. The mixture was thoroughly cooled in an ice bath, the solid collected by filtration, and dried to give 8.50 g (20.8 mmol, 70%) of the product **6**, R_f 0.19 (3:7 EtOAc:hexanes), as an off-white solid, mp 189-191 °C (lit.[2]mp 191-193 °C); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 1.70 (m, 4H), 3.29 (m, 2H), 3.55 (m, 2H), 7.30 (d, 2H, J = 9.1 Hz), 7.85 (s, 4, H), 8.19 (d, J = 9.1 Hz), 10.75 (s, 1H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 21.0, 27.5, 38.0, 51.0, 118.0, 122.5, 127.0, 132.0, 136.0, 142.0, 145.5, 169.0; ESI m/z 404 $(M+H)^+$; HRMS (ESI^+) calculated for $C_{18}H_{18}N_3O_6S$ 404.0910, observed 404.0916 $(M+H)^+$.

4-Nitro-2-(phenylamino)benzoic acid (8). To a solution of 2-chloro-4-nitrobenzoic acid (**7**, 20.0 g, 100 mmol) in DMF (200 mL) was addedaniline (18.6 g, 200 mmol) slowly with stirring at rt. The contents were degassed with argon for 15 min, and then anhydrous potassium carbonate (13.8 g, 100 mmol) was added, followed by Cu powder (2.0 g, 31.5 mmol) and CuI (0.2 g, 1.1 mmol). The mixture was stirred and heated at 120 °C, and progress of the reaction was monitored by TLC (1:1 EtOAc:hexanes). After 6 h, the mixture was cooled to rt and poured in to a solution of sodium hydroxide (25 g) in water (400 mL). Charcoal (1 g) was added, the mixture was stirred well with a glass rod, and left overnight. After filtration, the filtrate was extracted with diethyl ether (3 × 400 mL) and the organic extracts were discarded. Crushed ice was added to the aqueous phase, followed by a mixture of conc HCl (35 mL) and water (35 mL). The resulting precipitate was collected by filtration, washed with distilled water, and dried to give 20.0 g (77.5 mmol, 80%) of the product **8**, R_f 0.6 (7:3 EtOAc:hexanes), as a yellow solid. Crystallization from 95% ethanol gave an analytical sample, mp 232-234 °C (lit.[4] 232-234 °C); ^{1}H NMR (500 MHz, DMSO) δ 7.21 (t, J = 7.2 Hz, 1H), 7.35 (d, J = 7.8 Hz, 2H), 7.45 (t, J = 7.6 Hz, 2H), 7.51 (dd, J = 8.6, 1.4 Hz, 1H), 7.82 (d, J = 1.4 Hz, 1H), 8.12 (d, J = 8.7 Hz, 1H), 9.78 (s, 1H); ^{13}C NMR (125 MHz, DMSO) δ 107.4, 110.4, 117.1, 122.7, 124.6, 130.0, 133.4, 139.1, 147.5, 150.7, 168.5; ESI m/z 257 $(M-H)^+$; HRMS (ESI^+) calculated for $C_{13}H_9N_2O_4$ 257.0568, observed 257.0568 $(M-H)^+$.

3-Nitroacridin-9(10*H*)-one (9). Following the method of Sourdon, *et al.*,[5] compound **8** (20.0 g, 77.5 mmol) was added to hot **polyphosphoric acid**(200 g) at 120 °C. The resulting slurry was mixed well with a glass rod, and then stirred for 6 h at 120 °C. The slurry was then allowed to cool to 70 °C, was poured into a beaker containing crushed ice, the resulting mixture basified with concentrated ammonium hydroxide, and left overnight. The solid was collected by filtration, washed with distilled water (100 mL), and dried in a vacuum oven at 65 °C. The resulting solid was dissolved in DMF (100 mL), the solution heated to 120 °C for 2 h, cooled to rt, poured into crushed ice, and the mixture left overnight. The solid was collected by filtration, washed with distilled water (100 mL), and dried to give 12.0 g (50.0 mmol, 65%) of the product **9**, R_f 0.4 (3:7 EtOAc:hexanes), as a yellow solid, mp 394-396 °C dec. (lit.[4]mp>400 ºC); ^{1}H NMR (TFA + $CDCl_3$, 500 MHz) δ 7.90 (t, 1H, J = 7.3 Hz), 8.11 (d, 1H, J = 9.1 Hz), 8.30 (t, 1H, J = 7.3 Hz), 8.50 (d, 1H, J = 7.3 Hz), 8.80 (d, 1H, 7.3 Hz), 9.0 (m, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 111.5, 114.0, 116.3, 118.5, 120.0, 125.0, 129.0, 140.2, 140.6, 143.5, 153.0; ESI m/z 240 $(M+H)^+$; HRMS (ESI^+) calculated for $C_{13}H_8N_3O_3$ 240.0535, observed 240.0525 $(M+H)^+$.

9-Chloro-3-nitroacridine (10). Following the method of Iwamoto, *et al.*,[6] DMF (one drop) was added to a suspension of compound **9** (4.00 g, 16.7 mmol) in thionyl chloride (20 mL) and the mixture stirred at rt. Disappearance of the starting material was monitored by TLC using 3:7 EtOAc:hexanes. After 1 h, volatiles were evaporated under reduced pressure, and chloroform (100 mL) was added. The mixture was transferred into a beaker containing ice-water (100 mL), the two-phase mixture was stirred with glass rod, and then basified with ammonium hydroxide. The organic layer was separated, the aqueous layer was extracted with chloroform (100 mL), the combined organic layers washed with brine, dried over anhydrous sodium sulfate, filtered, and volatiles removed under reduced pressure to give 3.9 g (15 mmol, 90%) of the product **10**, R_f 0.9 (3:7 EtOAc:hexanes), as a yellow solid. This material was used for the next reaction without further purification. Crystallization from benzene gave an analytical sample, mp 210-211 °C (lit.[4]mp 208 °C); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.70 (t, 1H, J = 7.6 Hz), 7.90 (t, 1H, J = 8.0 Hz), 8.25 (d, 1H, J = 1.9 Hz), 8.32 (d, 1H, J = 8.7 Hz), 8.45 (d, 1H, J = 9.5 Hz), 8.55 (d, 1H, J = 2.3 Hz), 9.14 (s, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 119.6, 124.7, 125.4, 126.7, 127.0, 128.9, 130.3, 131.8, 141.7, 147.2, 148.9, 150.4; ESI m/z 258 $(M+H)^+$; HRMS (ESI^+) calculated for $C_{13}H_7N_2O_2Cl$ 258.0196, observed 258.0194 $(M+H)^+$.

4-(1,3-Dioxoisoindolin-2-yl)-N-(4-((3-nitroacridin-9-yl)amino)phenyl)butane-1-sulfonamide Hydrochloride (12). To a suspension of palladium on carbon (10% w/w, 125 mg) in glacial acetic acid (10 mL) in a Parr hydrogenation bottle was added a solution of**6** (0.25 g, 0.62 mmol) in glacial acetic acid (10 mL). (Note: To avoid fire, the palladium on carbon was placed in small flask, and glacial acetic acid was added down the walls of the flask until the catalyst was completely wetted. The suspension was then transferred into the hydrogenation bottle). Hydrogenation was carried out at 60 psi and progress of the reaction was monitored by TLC (7:3 EtOAc:hexanes). After 24 h, the mixture was filtered through a Celite pad and the catalyst rinsed with MeOH (20 mL) into a flask containing conc HCl (10 µL). The filtrate was immediately used for the next reaction.

To the solution of *N*-(4-aminophenyl)-4-(1,3-dioxoisoindolin-2-yl)butane-1-sulfonamide (**11**) and conc HCl (10 µL) in glacial acetic acid (20 mL) and MeOH (20 mL) from above was added a solution of **10** (320 mg, 1.2 mmol) in chloroform (20 mL). The mixture was heated at 60 °C and progress of the reaction was monitored by TLC (7:3 EtOAc:hexanes). After 2 h, volatiles were removed under reduced pressure, the residue loaded onto a silica gel column, and the column eluted with 3:7 EtOAc:hexanes to remove the less polar impurities. Further elution with CH_2Cl_2:MeOH:ammonium hydroxide 10:4:1 gave

fractions containing the product. Volatiles were removed under reduced pressure and 3% HCl in MeOH was added immediately. Volatiles were removed under reduced pressure to give 320 mg (0.50 mmol, 81%) of the product **12**, R_f 0.3 (7:3 EtOAc:hexanes), as brown solid, mp 279-281 °C; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 1.72 (m, 4H), 3.20 (t, 2H, J = 6.5 Hz), 3.58 (t, 2H, J = 6.5 Hz), 7.43 (m, 2H), 7.40 (d, 1H, J = 9.1 Hz), 7.42 (t, 1H, J = 3.2 Hz), 7.60 (d, 1H, J = 9.3 Hz), 7.80 (m, 4H), 7.85 (d, J = 9.3 Hz), 8.10 (m, 2H), 8.20 (d, 1H, J = 9.5 Hz), 8.4 (m, 2H), 8.82 (s, 1H), 10.2 (s, 1H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 20.6, 26.5, 36.7, 50.4, 113.2, 114.1, 116.0, 117.6, 120.1, 122.0, 125.0, 128.3, 125.9, 128.2, 131.5, 134.0, 134.2, 134.4, 136.0, 139.9, 140.4, 140.6, 141.0, 141.1, 149.8, 155.1, 167.9, 176.0, 188.0; HRMS (ESI$^+$) calculated for $C_{31}H_{26}N_5O_6S$ 596.1598, observed 596.1609 $(M+H)^+$.

4-Amino-N-(4-((3-nitroacridin-9-yl)amino)phenyl)butane-1-sulfonamide Dihydrochloride (1). Compound **12** (320 mg, 0.5 mmol) was suspended in aqueous methylamine (40% v/v, 10 mL) at rt. Stirring was continued and the progress of the reaction was monitored by TLC (CH_2Cl_2:MeOH:ammonium hydroxide 10:4:1). After 30 min, volatiles were removed under reduced pressure, the residue loaded onto a silica gel column, and the column eluted with CH_2Cl_2:MeOH:ammonium hydroxide 10:4:1. Volatiles were removed under reduced pressure and the residue dissolved in 3% HCl in MeOH (10 mL). Volatiles were removed under reduced pressure to give 140 mg (0.30 mmol, 60%) of the product **1**, R_f 0.53, as a red solid, mp 273-275 °C dec. (lit.[2]mp 275 ºC dec.); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.70 (m, 2H), 1.80 (m, 2H), 2.92 (t, 2H, J = 7.2 Hz), 3.27 (t, 2H, J = 7.2 Hz), 7.20 (m, 4H), 7.30 (m, 1H), 7.62 (d, 1H, J = 8.3 Hz), 7.81 (m, 3H), 7.95 (d, 1H, J = 8.3 Hz), 8.35 (d, 1H, J = 9.2 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 20.0, 25.3, 38.8, 50.5, 113.5, 114.6, 115.5, 116.6, 118.9, 122.2, 125.0, 125.3, 125.9, 127.8, 135.9, 136.7, 136.8, 138.9, 140.4, 150.2, 155.5; ESI m/z 466 $(M+H)^+$; HRMS (ESI$^+$) calculated for $C_{23}H_{24}N_5O_4S$ 466.1544, observed 466.1539 $(M+H)^+$.

***N*-(4-(*N*-(4-((3-Nitroacridin-9-yl)amino)phenyl)sulfamoyl)butyl)-5-((3a*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-d]imidazol-4-yl)pentanamide Hydrochloride (13)**. To a solution of compound **1** (116 mg, 0.25 mmol) in dry DMF (10 mL), was added DIPEA (184 µL, 1 mmol), followed by biotin-NHS[7] (127 mg, 0.375 mmol). The reaction mixture was stirred for 84 h. Progress of the reaction monitored by TLC using 9:1:1 DCM:MeOH:NH_4OH as eluant, the solvent was evaporated under reduced pressure. The residue was dissolved in 10% methanol in DCM, loaded onto a silica gel column and eluted with (9:1:1 DCM:MeOH:NH_4OH). The fractions containing the product were evaporated under reduced pressure. As soon as evaporated the solvent, the residue was dissolved in 3% HCl in methanol (5 mL). The solvent was evaporated under reduced pressure to give 70 mg (0.1 mmol, 40%) of the product **13**, R_f 0.5 (1:9:1 MeOH, DCM, NH_4OH) as brown red solid, mp 168-170 ˚C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.27 (m, 2H), 1.30-1.60 (m, 7H), 1.73 (m, 2H), 2.08 (t, 2H, J = 1Hz), 2.53 (d, 1H, J = 1Hz), 2.75-2.90 (m, 2H), 3.0 (m, 3H), 3.10 (t, 2H, J = 1Hz), 3.20 (m, 2H), 3.53 (s, 1H), 4.20 (m, 1H), 4.38 (m, 1H), 7.28-7.34 (m, 4H), 7.38 (m, 1H), 7.86-7.97 (m, 3H), 8.07 (d, 1H, J = 8Hz), 8.30 (d, 1H, J = 8Hz), 8.64 (s, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 21.9, 26.8, 28.8, 29.3, 29.6, 36.7, 39.4, 41.0, 52.2, 56.8, 61.6, 63.3, 115.3, 115.9, 117.4, 117.9, 120.4, 122.2, 126.3, 126.8, 127.2, 129.5, 136.9, 137.9, 139.7, 141.0, 142.5, 152.1, 157.3, 166.0, 176.4. ESI m/z = 692 $(M+H)^+$. HRMS (ESI$^+$): calcd for $C_{33}H_{38}N_7O_6S_2$ 692.2319, observed 692.2311 $(M + H)^+$.

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