Ultrasound-assisted Green Synthesis and Structure–Activity Relationship (SAR) Studies of Novel N-Substituted-2-[2-(3-fluoro-9H-carbazol-9-yl) acetyl] hydrazine-1-carbothioamide Derivatives with Potential Human Breast Cancer Proliferation Activity

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ABSTRACT

Breast cancer is one of the leading causes of cancer-related deaths worldwide, and drug resistance poses a substantial challenge to effective breast cancer treatment. This experimental study presents a novel route for the synthesis of N-substituted-2-[2-(3-fluoro-9H-carbazol-9-yl) acetyl] hydrazine-1-carbothioamide derivatives using an ultrasound-assisted green methodology, yielding the synthesised derivatives with excellent efficiency. This approach has significant advantages over traditional methods and includes fast reaction time, environmentally friendly, good selectivity, and high yields. Characterization of the derivatives was done using IR, mass, 1H and 13C NMR spectroscopy. The anti-proliferative activity of synthesised compounds was assessed against MCF-7 breast cancer cell lines. A structure-activity relationship (SAR) study revealed that the compounds containing a benzoyl and benzyl substituent displayed significant activity compared to Etoposide as a standard drug.

KEYWORDS

ultrasonication, 3-fluoro-9H-carbazole derivatives, thiosemicarbazide linker, breast cancer, and anti-proliferation activity

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INTRODUCTION

The development of chemotherapeutic drugs is important for the prevention and management of many illnesses.[1] Microorganisms can develop resistance to certain chemotherapeutic treatments, and resistant strains of microorganisms present substantial hurdles in treating microbial diseases.[2] As a result, the search for a new antimicrobial agents continue with significant efforts devoted to discovering new antibiotics or chemical substances with potent antimicrobial properties that may serve as chemotherapeutic agents.[3] N-Heterocyclic compounds are an important class of molecules that have attracted much attention in numerous biological studies since they act as scaffolds for the synthesis of novel therapeutic possibilities.[4] Carbazoles and its derivatives are an important class of nitrogen-containing aromatic heterocyclic compounds, that have a large π-conjugated system, desirable electronic and charge-transport properties, and the ability to readily incorporate different functional groups into the structurally rigid carbazolyl ring.[5] Due to their wide range of biological and pharmacological actions, carbazole-based derivatives have a variety of potential uses in the field of medicinal chemistry.[6] These substances have antibacterial,[7] anticancer,[8] antioxidant,[9] anti-inflammatory,[10] and antidiabetic activity,[11] which underscore their potential use in a number of therapeutic areas. However, in synthetic chemistry, the thiosemicarbazide functional group is essential for creating a variety of heterocyclic molecules.[12] Thiosemicarbazide derivatives are known for the biological activities listed above as well as analgesic, anticonvulsant activity, and antiallergic properties.[13]

Synthetic medicinal chemists are motivated to develop new synthetic carbazole derivatives that are therapeutically effective and similar to natural carbazoles. Several important commercially available drugs: Ellipticine, Alectinib, Carvedilol, Carazolol, Carprofen, and Frovatriptan (Figure 1), include the carbazole moiety.[14] Ellipticine, a naturally occurring alkaloid, was identified as an anticancer agent in 1959. It is regarded as one of the first effective therapeutic carbazole analogs for the treatment of cancer and was isolated from the leaves of Ochrosia elliptica.[15] The FDA authorized the use of Alexinib in 2015 to treat advanced non-small cell lung cancer.[16] Frovatriptan is an orally administered triptan drug approved for the acute treatment of adult migraine, with or without aura.[17] Carvedilol (Dilatrend) inhibits beta-1, beta-2 and alpha-1-adrenoceptors, and has antioxidant and antiproliferative properties.[18] Carazolol is a beta-1/beta-2 adrenoceptor

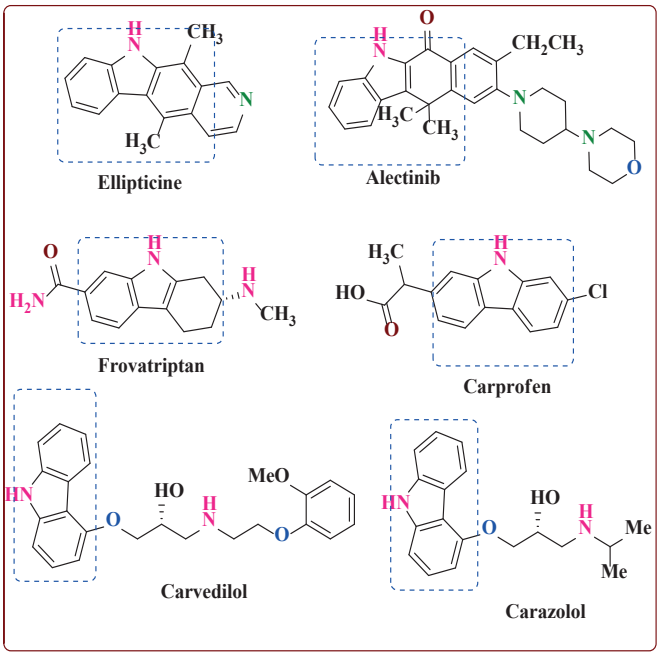


Figure 1. Approved drugs containing notable 9H-carbazoles

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antagonist of high potency used in the treatment of hypertension,[19] Carprofen is a nonsteroidal anti-inflammatory drug.[20]

A hybrid compound is an artificial combination of two or more pharmacophores that can be classified as a recognized agent capable of eliciting the desired activity.[21] Antibiotics that can overcome drug resistance, increase binding affinity, and amplify action could be produced using such artificial scaffolds.[22] Antibacterial efficacy may be maintained against organisms that remain resistant to the drug's ingredients, however this is unpredictable.[23] The introduction of additional physicochemical properties during the hybridization process has the potential to affect the pharmacological spectrum of the hybrid. For example, the combination of two compounds may enhance their efficacy or potentially provide the resulting hybrid agent with new antibacterial properties.[24] The 9H-carbazole was chosen as the primary skeleton, while the thiosemicarbazide functional group serves as the side chain. Data analysis suggests potential therapeutic value, particularly in the development of novel compounds with diverse biological activities, such as anticancer, antibacterial, and antioxidant properties.[25]

The ultrasonication (sonochemical process) approach is a key technique of green synthetic chemistry, providing a versatile pathway for synthesising a variety of bioactive molecules. Numerous organic processes can be performed under mild conditions using ultrasonic irradiation, giving high yields in a short amount of time.[26] Acoustic cavitation is a physical phenomenon that enables particular activation when ultrasound is used in chemical reactions in solution. Cavitation is a process where the attraction forces of molecules in the liquid phase are destroyed by mechanical stimulation. In this study we present our ongoing efforts to develop practical synthetic methods for the synthesis of novel hybrid analogues based on 9H-carbazole linked acetylthiosemicarbazide derivatives that exhibit biological activity. Our synthetic method reveals a high-yield, simple sonochemical synthesis that produces breast cancer proliferative agents.

EXPERIMENTAL

Materials and Instrumentation

The solvents and reagents used were of analytical purity and procured from readily accessible markets. The LDMP-A20 Automatic Melting Point instrument was used to determine the compounds' melting points, and the results presented are without correction. To verify the compounds' purity, aluminium plates covered with silica gel 60 F254 (Merck) were used. Hexane/ethyl acetate and dichloromethane/ethyl acetate were the solvent systems used in thin-layer chromatography (TLC). Reactions were tracked using a Camag UV lamp at 254 nm and 366 nm. TLC and UPLC/MS-TOF tests verified compound purity. ^{13}C NMR (100 MHz) is referenced to DMSO-d6. The ^{1}H NMR spectra were obtained on a Bruker 400-MHz spectrometer and presented in ppm (δ) relative to tetramethyl silane (TMS) as the internal standard. Chemical changes ppm is reported in ppm (parts per million). Coupling constants were recorded in Hertz (Hz). HRMS spectra of the compounds were obtained from methanol solutions using positive-ion (ESI+) electrospray ionization on a Waters LCT Premier XE UPLC/MS/TOF system equipped with the Mass Lynx 4.1 software. The stationary phase was an Aquity BEH C18 column (2.1 × 100 mm, 1.7 µM, flow rate: 0.3 mL/min), while the mobile phase was a CH_3CN/H_2O gradient solvent system (1–90%) with formic acid (0.1%). The Mestre Nova 12 program was used for spectral verification, LC-MS data processing, and NMR FID. ChemDraw19 software was used to draw the 2D molecules.

General procedure

Synthesis of ethyl 2-(3-fluoro-9H-carbazole-9-yl) acetate (2)

Based on previous reports,[27-28] (step 1) ethyl-2-(3-fluoro-9H-carbazol-9-yl) acetate (930 mg) (2), containing an ester functional group on the nitrogen atom, was prepared by reacting commercially purchased ethyl 2-[(2-phenyl-4-fluoro-phenyl)-amino]acetate (3.9 mmol, 1000 mg) (1) with 2.5 mol% Pd $(OAc)_2$ (0.025 mmol, 5.6 mg) as the catalyst and H_2O_2 (0.1 mL) as the oxidant in a cyrene (10 mL) solvent system with air bubbling at 110°C. The mixture was mechanically stirred for six hours to complete the reaction, and TLC was used to track the progress. The crude product (930 mg) was obtained by gradually adding the mixture dropwise to 200 mL of ice-cold water after the reaction was complete. Excellent yields of 93% were obtained by filtering, drying, and recrystallizing the resultant solid in isopropyl alcohol (10 mL). The synthesised compound (2) was elucidated by ^{1}H NMR, ^{13}C NMR, and HRMS/elemental analysis and is described in the results and discussion section.

Synthesis of 1-(3-fluoro-9H-carbazole-9-yl)-2-hydrazinylethanone (3)

In the second step, compound (2) (3.95 mmol, 1000 mg) was reacted with hydrazine monohydrate (80% in water, 3 mmol, 0.15 mL) in ethanol (10 mL) at 80°C for 6 h while stirred mechanically to produce the hydrazide derivative 1-(3-fluoro-9H-carbazole-9-yl)-2-hydrazinylethanone (81 mg) (3). Following the completion of compound (2) in the reaction medium, solvent evaporation on a rotary evaporator in vacuo was monitored by TLC. The crude product was dissolved in 10 mL of methanol and added dropwise to 200 mL of ice-cold water. Vacuum filtration was used to dry the resultant solid. By recrystallizing the crude product from isopropyl alcohol, good yields of 81% were achieved. The synthesised compound (3) was characterized by ^{1}H NMR, ^{13}C NMR, and HRMS/elemental analysis, and the results are described in the results and discussion section.

Synthesis of new N-substituted-2-[2-(3-fluoro-9H-carbazol-9-yl) acetyl] hydrazine-1-carbothioamide derivatives 4(a-r)

N-substituted-2-[2-(3-fluoro-9H-carbazol-9-yl)acetyl]hydrazine-1-carbothioamide as novel derivatives **4(a-r)** were prepared in high to excellent yields by reacting compound (3), 1-(3-fluoro-9H-carbazole-9-yl)-2-hydrazinylethanone (4.18 mmol, 1000 mg) with different substituted isothiocyanates **4a** (3.28 mmol)**, 4b** (3.24 mmol), **4c** (3.48 mmol), **4d** (4.01 mmol), **4e** (3.99 mmol), **4f** (4.24 mmol), **4g** (4.16 mmol), **4h** (3.84 mmol), **4i** (4.13 mmol), **4j** (4.20 mmol), **4k** (4.18 mmol), **4l** (3.64 mmol), **4m** (4.24 mmol), **4n** (4.19 mmol), **4o** (3.13 mmol), **4p** (4.03 mmol), **4q** (3.39 mmol), and **4r** (4.01 mmol) in 10 mL of ethanol at 80°C using an ultrasonic bath (40 kHz, 250 W).The reaction was completed in 35-40 minutes using ultrasonication while 2-3 hours is required, using the conventional method. The reaction progress was monitored by TLC. In addition, the yields and purities of the prepared compounds were compared to those obtained using the traditional approach. Column chromatography was used to purify all crude compounds, and ^{1}H NMR, ^{13}C NMR, and HPLC spectra were obtained for structural elucidation. High-resolution mass spectrometry was used to obtain the mass spectra. The eighteen distinct chemical derivatives that were produced and described, were used in the anti-proliferation activity study and structure–activity evaluation.

Spectral data of new N-substituted-2-[2-(3-fluoro-9H-carbazol-9-yl) acetyl] hydrazine-1-carbothioamide derivatives 4(a-r)

N-(4-chlorophenyl) 2-[2-(3-fluoro-9H-carbazol-9-yl) acetyl] hydrazine-1-carbothioamide (4a)

Appearance: White crystalline solid; Mp: 235–237 °C, ($R_{f\ =}$ 0.48, EtOAc: DCM (9:1), Yield: 90%. 3407, 3257 (N–H stretching), 3058 (aromatic C–H), 2983, 2940 (aliphatic C–H), 2764, 2726 (weak overtone/combination bands), 2509, 2387 (broad bands), 1626 (C=O, amide), 1606, 1586 (aromatic C=C), 1507, 1451 (ring vibrations), 1400, 1343 (C–N stretching), 1304, 1253, 1215 (C=S and C–N), 1101, 1021 (C–N/C–S), 991, 949, 881 (aromatic C–H bending), 844, 782, 755, 724, 701 (substituted aromatic ring), 638, 620 (C–Cl). ^{1}H NMR

(400 MHz, DMSO-d_6) δ: 10.49 (s, 1H), 9.75 (d, J = 28.4 Hz, 2H), 8.16 (d, J = 7.7 Hz, 2H), 7.60 (d, J = 8.2 Hz, 2H), 7.53 (d, J = 8.8 Hz, 2H), 7.50–7.39 (m, 4H), 7.23 (t, J = 7.4 Hz, 2H), 5.17 (s, 2H). ^{13}C NMR (100 MHz, DMSO) δ: 171.38 (C=S), 167.88 (C=O), 141.20, 141.06 (Ar-Cq), 138.50 (Ar-Cq, C–F/C–Cl), 128.62, 126.14, 126.02, 122.73, 120.66, 120.62, 119.59, 119.44 (Ar-CH), 109.95, 109.83 (carbazole-CH), 44.54 (CH_2). HRMS (ESI^+) m/z: $[M+H]^+$ calculated for $C_{21}H_{16}ClFN_4OS$, 426.0717; found, 426.1955.

N-(3-methoxyphenyl)2-[2-(3-fluoro-9H-carbazol-9-yl) acetyl] hydrazine-carbothioamide (4b)

Appearance: White solid isolated; Mp: 218–220°C; ($R_{f=}$ 0.42, EtOAc: DCM (9:1), Yield: 84%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.53 (s, 1H), 9.78 (s, 2H), 8.18 (d, J = 7.7 Hz, 2H), 7.61 (d, J = 8.2 Hz, 2H), 7.51–7.41 (m, 2H), 7.34–7.13 (m, 5H), 7.05 (d, J = 8.0 Hz, 1H), 6.80 (d, J = 8.3 Hz, 1H), 5.19 (s, 2H), 3.77 (s, 3H). ^{13}C NMR (100 MHz, DMSO) δ: 171.39 (C=S), 167.24 (C=O), 159.57 (Ar–O–CH_3 ipso), 141.08, 140.60 (Ar-Cq) 129.46, 126.13, 126.09, 126.01, 122.74, 120.65, 120.63, 119.58, 119.45, 119.42 (Ar-CH), 109.97, 109.83, (carbazole-CH), 55.61, 44.55 (CH_2). **Elemental analysis** for $C_{22}H_{19}FN_4O_2S$ (M = 422.12): calculated (%) C 62.54, H 4.53, F 4.50, N 13.26, O 7.57, S 7.59; found (%) C 62.45, H 4.60, F 4.70, N 13.50, O 7.50, S 7.65.

N-(3-morpholinopropyl)2-[2-(3-fluoro-9H-carbazol-9-yl)acetyl]-hydrazine-1-carbothioamide (4c)

Appearance: White solid isolated; Mp: 189–191 °C Yield: 92%. FTIR (KBr, νmax, cm^{-1}): 3409 (N–H stretching), 3057 (aromatic C–H), 2956, 2929, 2869 (aliphatic C–H), 1732 (C=O, amide), 1608, 1507 (aromatic C=C), 1449, 1417 (C–N), 1380, 1341 (C–N/C=S), 1304, 1262, 1213 (C=S and C–N), 1169 (C–O–C, morpholine), 1100 (C–N/C–O), 994, 935, 908 (aromatic C–H bending), 785, 751, 723 (substituted aromatic ring), 658 (C–S). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.75 (s, 1H), 8.21 (d, J = 7.7 Hz, 2H), 7.68 (d, J = 8.2 Hz, 2H), 7.55–7.39 (m, 2H), 7.27 (t, J = 7.4 Hz, 2H), 5.86 (s, 2H), 3.97–3.77 (m, 2H), 3.47 (t, J = 4.6 Hz, 4H), 2.08 (d, J = 4.7 Hz, 4H), 1.78 (t, J = 6.8 Hz, 2H), 1.21–1.13 (m, 2H). ^{13}C NMR (100 MHz, DMSO) δ: 167.82 (C=S), 148.97 (C-3, C–F), 140.44 (C-4a/C-9a), 123.00 (C-1/C-8), 66.52 (OCH_2, morpholine), 55.01-53.35 (NCH_2, morpholine), 42.63, 38.71, 24.13 (CH_2, propyl). HRMS (ESI^+) m/z: $[M + H]^+$ calculated for $C_{22}H_{26}FN_5O_2S$, 443.1791; found, 443.1693.

N-(naphthalene-1-yl)2-[2-(3-fluoro-9H-carbazol-9-yl)acetyl]-hydrazine-1-carbothioamide (4d)

Appearance: White solid isolated; Mp: 222–224 °C; Yield: 85%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.66 (s, 1H), 9.99 (s, 1H), 9.81 (s, 1H), 8.20 (d, J = 7.7 Hz, 2H), 7.99 (d, J = 8.2 Hz, 1H), 7.91 (dd, J = 8.4, 5.5 Hz, 2H), 7.67–7.52 (m, 5H), 7.52–7.40 (m, 4H), 7.25 (t, J = 7.4 Hz, 2H), 5.22 (s, 2H). ^{13}C NMR (100 MHz, DMSO) δ: 171.26 (C=S), 168.07 (C=O), 141.14 (C-3, C–F), 134.25, 128.40, 127.49, 126.58, 126.45, 126.13, 126.05, 125.91, 124.05, 122.77, 120.68, 119.59, 119.46(Ar-CH), 109.95 (carbazole-CH), 44.51 (CH_2). **Elemental analysis** for $C_{25}H_{19}FN_4OS$ (M = 442.13): calculated (%) C 67.86, H 4.33, F 4.29, N 12.66, O 3.62, S 7.25; found (%) C 67.76, H 4.39, F 4.40, N 12.56, O 3.52, S 7.35.

N-(4-nitrophenyl)2-[2-(3-fluoro-9H-carbazol-9-yl)acetyl]-hydrazine-1-carbothioamide (4e)

Appearance: White solid isolated; Mp: 235–237 °C; Yield: 94%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.01–9.82 (m, 3H), 8.38–8.09 (m, 4H), 7.93 (s, 2H), 7.72–7.53 (m, 2H), 7.46 (t, J = 7.5 Hz, 2H), 7.23 (td, J = 7.4, 3.3 Hz, 2H), 5.20 (s, 2H). ^{13}C NMR (100 MHz, DMSO) δ: 171.34 (C=S), 169.6 (C=O), 146.01 (Ar-Cq),141.19, 141.04, (C-3, C–F), 126.17, 126.03, 125.14, 124.30, 122.74, 120.67, 120.15, 119.61, 119.45, 109.97(Ar-Cq), 109.88,(carbazole-CH),44.55 (CH_2). **Elemental analysis** for $C_{21}H_{16}FN_5O_3S$ (M = 437.10): calculated (%) C 57.66, H 3.69, F 4.34, N 16.01, O 10.97, S 7.33; found (%) C 57.86, H 3.65, F 4.30, N 16.10, O 10.90, S 7.25.

N-(4-methoxyphenyl)2-[2-(3-fluoro-9H-carbazol-9-yl)acetyl]-hydrazine-1-carbothioamide (4f)

Appearance: White solid isolated; Mp: 210–212 °C; Yield: 95%. FTIR (KBr, νmax, cm^{-1}): 3408 (N–H stretching), 3067 (aromatic C–H), 2976, 2932, 2857 (aliphatic C–H), 1883 (overtones/combination band), 1739 (C=O, amide I), 1638 (C=O/C=N, amide II contribution), 1604, 1507 (aromatic C=C), 1448, 1380 (C–N stretching), 1341, 1304 (C–N/C=S stretching), 1262, 1212 (C–O–C, methoxy group), 1150 (C–O/C–N), 1101 (C–F/C–O vibrations), 978 (Ar–H bending), 847, 787, 752, 724, 699 (substituted aromatic C–H out-of-plane bending), 663, 619 (C–S stretching). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.50 (s, 1H), 9.66 (d, J = 6.1 Hz, 2H), 8.17 (d, J = 7.7 Hz, 2H), 7.59 (d, J = 8.2 Hz, 2H), 7.46 (ddd, J = 8.2, 7.1, 1.3 Hz, 3H), 7.33–7.20 (m, 4H), 6.94 (d, J = 8.9 Hz, 2H), 5.17 (s, 2H), 3.77 (s, 3H). ^{13}C NMR (100 MHz, DMSO) δ: 171.37 (C=S), 167.72 (C=O), 157.41, 141.10, 141.07 **(C-3, C–F)**, 132.31, 132.27, 126.13, 126.01, 122.74, 122.71, 120.67, 120.64, 119.56, 119.42, 113.92, 109.95, 109.82 (carbazole-CH), 55.73, 55.70 (OCH_3), 44.55, 44.51, 40.65, 40.59, 40.44, 40.38, 40.23, 40.17, 40.02, 39.96, 39.82, 39.75, 39.61, 39.54, 39.40, 39.33 (CH_2). **Elemental analysis** for $C_{22}H_{19}FN_4O_2S$ (M = 422.12): calculated (%) C 62.54, H 4.53, F 4.50, N 13.26, O 7.57, S 7.59; found (%) C 62.60, H 4.50, F 4.40, N 13.30, O 7.60, S 7.50.

N-(4-bromophenyl)2-[2-(3-fluoro-9H-carbazol-9-yl)acetyl]-hydrazine-1-carbothioamide (4g)

Appearance: White solid isolated; Mp: 255–257 °C; Yield: 89%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.47 (s, 1H), 9.75 (d, J = 11.0 Hz, 2H), 8.16 (d, J = 7.7 Hz, 2H), 7.58 (dd, J = 19.9, 8.3 Hz, 4H), 7.52–7.37 (m, 4H), 7.24 (t, J = 7.4 Hz, 2H), 5.17 (s, 2H). ^{13}C NMR (100 MHz, DMSO) δ: 171.35 (C=S), 167.75 (C=O), 141.08 **(C-3, C–F)**, 138.96, 131.54, 126.14, 126.01, 122.75, 120.64, 120.61, 119.58, 109.95, (carbazole-CH) 44.57 (CH_2). **Elemental analysis** for $C_{21}H_{16}BrFN_4OS$ (M = 470.02): calculated (%) C 53.51, H 3.42, Br 16.95, F 4.03, N 11.89, O 3.39, S 6.80; found (%) C 53.55, H 3.47, Br 16.90, F 4.08, N 11.84, O 3.44, S 6.75.

N-(phenyl)2-[2-(3-fluoro-9H-carbazol-9-yl) acetyl]-hydrazine-1-carbothioamide (4h)

Appearance: White solid isolated; Mp: 235–237 °C; Yield: 88%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.52 (s, 1H), 9.86–9.67 (m, 2H), 8.17 (d, J = 7.7 Hz, 2H), 7.61 (d, J = 8.1 Hz, 2H), 7.47 (t, J = 8.1 Hz, 4H), 7.39 (q, J = 7.6 Hz, 2H), 7.24 (tt, J = 7.0, 3.3 Hz, 3H), 5.18 (d, J = 2.1 Hz, 2H). ^{13}C NMR (100 MHz, DMSO) δ: 184.16 (C=S), 167.77 (C=O), 141.09, (C - 3, C – F) 139.50, 128.70, 126.14, 126.02,122.75, 120.65, 119.58 (Ar-CH), 109.96 (carbazole-CH), 44.56, 40.64, 40.43, 40.22,40.01, 39.80, 39.59, 39.39 (CH_2). **Elemental analysis** for $C_{21}H_{17}FN_4OS$ (M = 392.11): calculated (%) C 64.27, H 4.37, F 4.84, N 14.28, O 4.08, S 8.17; found (%) C 64.30, H 4.32, F 4.89, N 14.24, O 4.13, S 8.12.

N-(4-trifluoromethylphenyl)2-[2-(3-fluoro-9H-carbazol-9-yl) acetyl]-hydrazine-1-carbothioamide (4i)

Appearance: White solid isolated; Mp: 177–179 °C; Yield: 92%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.17 (d, J = 7.7 Hz, 2H), 7.81 (d, J = 8.5 Hz, 2H), 7.73 (d, J = 8.4 Hz, 2H), 7.62 (d, J = 8.2 Hz, 2H), 7.47 (ddd, J = 8.3, 7.0, 1.2 Hz, 2H), 7.24 (t, J = 7.4 Hz, 2H), 5.19 (s, 2H). ^{13}C NMR (100 MHz, DMSO) δ: 171.38(C=S), 167.88(C=O), 143.35(Ar-CF3), 141.21, 141.07(C-3, C–F),128.84, 126.16, 126.03, 125.83, 123.45, 122.75, 120.66,119.62, 119.60 (Ar-CH), 109.96 (carbazole-CH), 44.56 (CH_2). **Elemental analysis** for $C_{22}H_{16}F_4N_4OS$ (M = 460.10):

calculated (%) C 57.39, H 3.50, F 16.50, N 12.17, O 3.47, S 6.96; found (%) C 57.30, H 3.59, F 16.59, N 12.00, O 3.44, S 6.90.

N-(cyclohexyl)2-[2-(3-fluoro-9H-carbazol-9-yl) acetyl]-hydrazine-1-carbothioamide (4j)

Appearance: White solid isolated; Mp: 211–213 °C; Yield: 78%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.19 (s, 1H), 9.32 (d, J = 107.9 Hz, 1H), 8.16 (d, J = 7.7 Hz, 2H), 7.59 (d, J = 8.3 Hz, 2H), 7.46 (q, J = 7.8, 6.2 Hz, 3H), 7.23 (t, J = 7.4 Hz, 2H), 5.13 (s, 2H), 2.03–1.50 (m, 6H), 1.25 (d, J = 10.3 Hz, 5H). ^{13}C NMR (100 MHz, DMSO) δ: 171.47 (C=S), 167.47 (C=O), 141.15,141.04 (Ar–Cq–N), 126.12, 126.02, 122.73, 122.71, 120.64, 119.57, 119.55 (Ar–CH), 109.95 (carbazole-CH), 53.26 (N–CH), 44.47 ($COCH_2$), 32.40, 25.65, 25.26 (cyclohexyl CH_2). **Elemental analysis** for $C_{21}H_{23}FN_4OS$ (M = 398.15): calculated (%) C 63.29, H 5.82, F 4.77, N 14.06, O 4.01, S 8.05; found (%) C 63.20, H 5.87, F 4.87, N 14.16, O 4.10, S 8.15.

N-(p-tolyl)2-[2-(3-fluoro-9H-carbazol-9-yl) acetyl]-hydrazine-1-carbothioamide (4k)

Appearance: White solid isolated; Mp: 180–182 °C; Yield: 81%. FTIR (KBr, νmax, cm^{-1}): 3409 (N–H stretching), 3058 (aromatic C–H), 2926, 2855 (aliphatic C–H, CH_3), 1699 (C=O, amide I), 1610, 1507 (aromatic C=C), 1481, 1449 (C–N stretching / ring vibrations), 1417, 1380 (CH_3 bending), 1340, 1305 (C–N/C=S stretching), 1262, 1214 (C–N and C=S stretching), 1102, 1051 (C–F/C–N vibrations), 992, 912 (aromatic C–H bending), 787, 753, 724, 699 (substituted aromatic C–H out-of-plane bending), 642, 618 (C–S stretching). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.45 (s, 1H), 9.61 (d, J = 10.4 Hz, 2H), 8.16 (d, J = 7.7 Hz, 2H), 7.60 (d, J = 8.2 Hz, 2H), 7.46 (t, J = 7.5 Hz, 2H), 7.34 (d, J = 8.0 Hz, 2H), 7.30–7.13 (m, 4H), 5.17 (s, 2H), 2.31 (s, 3H). ^{13}C NMR (100 MHz, DMSO) δ 171.38 (C=S), 167.75 (C=O), 141.22, 141.08 (C-3, C–F), 136.89, 135.10, (Ar–Cq–N) 129.16, 126.13, 126.02, 122.73, 120.66, 120.62, 119.57, 119.43 (Ar–CH), 109.95, 109.82 (carbazole-CH), 44.53, 21.05 (CH_3). HRMS (ESI^+) m/z: $[M + H]^+$ calcd for $C_{22}H_{19}NF_4OS$, 406.1264; found, 406.1298.

N-(benzamide)2-[2-(3-fluoro-9H-carbazol-9-yl) acetyl] hydrazine-1-carbothiomide (4l)

Appearance: White solid isolated; Mp: 189–191°C; ($R_{f=}$ 0.49, EtOAc: DCM (9:1), Yield: 75%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.70 (s, 1H), 11.57 (d, J = 108.8 Hz, 2H), 8.17 (d, J = 7.7 Hz, 2H), 7.95 (d, J = 7.7 Hz, 2H), 7.65 (d, J = 8.1 Hz, 3H), 7.49 (dt, J = 16.4, 7.8 Hz, 4H), 7.24 (t, J = 7.6 Hz, 2H), 5.31 (s, 2H). ^{13}C NMR (101 MHz, DMSO) δ: 177.70 (C=S), 168.51, 165.48 (C=O), 141.03 (C-3, C–F), 133.64, 132.24, 129.33, 129.20, 128.91, 128.78, 126.22, 122.74, 120.67, 119.66, 119.63 (Ar–CH), 109.99 (carbazole-CH), 44.21 (CH_3). HRMS (ESI^+) m/z: $[M + H]^+$ calcd for $C_{22}H_{17}FN_4O_2S$, 420.1056; found, 420.1133.

N-(benzyl)2-(2-(3-fluoro-9H-carbazol-9-yl) acetyl)-hydrazine-1-carbothioamide (4m)

Appearance: White solid isolated; Mp: 169–171 °C; Yield: 86%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.32 (s, 1H), 9.43 (s, 1H), 8.16 (d, J = 7.7 Hz, 2H), 7.55 (d, J = 8.2 Hz, 2H), 7.51–7.37 (m, 3H), 7.32 (d, J = 4.3 Hz, 4H), 7.29–7.19 (m, 3H), 5.12 (s, 2H), 4.81 (d, J = 6.0 Hz, 2H). ^{13}C NMR (100 MHz, DMSO) δ: 171.29 (C=S), 167.78 (C=O), 141.09, 141.05 (C-3, C–F), 139.62, 128.70, 128.60, 128.07, 127.36, 127.15, 126.11, 126.02, 122.71, 120.65, 120.62, 119.56, 119.46 (Ar–CH), 109.90, 109.67 (carbazole-CH), 47.20, 44.43 (CH_3). HRMS (ESI^+) m/z: $[M + H]^+$ calcd for $C_{22}H_{19}FN_4OS$, 406.1264; found, 406.1178.

N-(bicyclo[2.2.1]heptan-2-yl)2-(2-(3-fluoro-9H-carbazol-9-yl) acetyl)-hydrazine-1-carbothioamide (4n)

Appearance: White solid isolated; Mp: 199–201 °C; Yield: 73%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.20 (s, 1H), 9.31 (d, J = 88.8 Hz, 1H), 8.16 (d, J = 7.8 Hz, 2H), 7.60 (d, J = 8.2 Hz, 2H), 7.49–7.42 (m, 2H), 7.27–7.20 (m, 2H), 5.14 (s, 2H), 3.93 (s, 1H), 2.28–2.15 (m, 2H), 1.70–1.61 (m, 1H), 1.54–1.37 (m, 3H), 1.32 (d, J = 10.5 Hz, 2H), 1.25–1.04 (m, 4H). ^{13}C NMR (101 MHz, DMSO) δ: 171.44 (C=S), 167.46 (C=O), 141.18, 141.02 **(C-3, C–F)**, 126.13, 126.00, 122.75, 122.73, 122.71, 120.66, 120.62, 119.58, 119.42 (Ar–CH), 109.91, 109.77 (carbazole-CH), 57.69 (bridgehead-CH), 44.47, 42.19 (N-CH_2), 35.75, 35.41, 28.37, 26.50 (CH_3). HRMS (ESI^+) m/z: $[M + H]^+$ calcd for $C_{22}H_{23}FN_4OS$, 410.1577; found, 410.1523.

N-phenethyl 2-[2-(3-fluoro-9H-carbazol-9-yl) acetyl] hydrazine-1-carbothioamide (4o)

Appearance: White solid isolated; Mp: 170–172 °C; Yield: 84%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.26 (s, 1H), 9.45 (d, J = 83.8 Hz, 1H), 8.16 (d, J = 7.7 Hz, 2H), 8.08 (s, 1H), 7.57 (d, J = 8.2 Hz, 2H), 7.52–7.40 (m, 3H), 7.38–7.15 (m, 7H), 5.11 (s, 2H), 3.86–3.57 (m, 2H), 2.86 (dd, J = 9.1, 6.4 Hz, 2H). ^{13}C NMR (100 MHz, DMSO) δ: 171.44 (C=S), 167.73 (C=O), 159.32, 141.05 (C-3, C–F), 139.65, 129.10, 128.91, 126.65, 126.13, 122.71, 120.66, 119.57 (Ar–CH), 109.91 (carbazole-CH), 45.78, 44.44 (N-CH_2), 35.37 (CH_3). **Elemental analysis** (%) calculated (%) for $C_{23}H_{21}FN_4OS$: C 65.69, H 5.03, F 4.52, N 13.32, O 3.80, S 7.63; found: C 65.60, H 5.10, F 4.59, N 13.40, O 3.87, S 7.67.

N-(isopropyl)2-[2-(3-fluoro-9H-carbazol-9-yl) acetyl]-hydrazine-1-carbothioamide (4p)

Appearance: White solid isolated; Mp: 186–188 °C; Yield: 90%. FTIR (KBr, νmax, cm^{-1}): 3415, 3328 (N–H stretching), 3060 (aromatic C–H), 2977, 2939, 2863 (aliphatic C–H, isopropyl), 1895, 1785 (overtones/combination bands), 1723 (C=O, amide I), 1600, 1505 (aromatic C=C), 1446, 1379 (CH_3 bending / C–N stretching), 1341, 1300 (C–N/C=S stretching), 1259, 1213 (C–N and C=S stretching), 1094, 1023 (C–N/C–F vibrations), 989, 935, 848 (aromatic C–H bending), 788, 753, 722, 700 (substituted aromatic ring out-of-plane bending), 650, 618 (C–S stretching). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.18 (s, 1H), 9.32 (d, J = 117.8 Hz, 1H), 8.16 (d, J = 7.7 Hz, 2H), 7.65–7.39 (m, 5H), 7.23 (t, J = 7.4 Hz, 2H), 5.13 (s, 2H), 4.55–4.21 (m, 1H), 1.15 (d, J = 6.5 Hz, 6H). ^{13}C NMR (100 MHz, DMSO) δ: 171.44 (C=S), 167.51 (C=O), 141.14, 141.05 (C-3, C–F), 126.12, 126.02, 122.72, 120.64, 119.56, 119.50 (Ar–CH), 109.95, 109.93, 109.75 (carbazole-CH), 46.22, 44.46, 44.41 (N-CH_2), 22.44, 22.40 (CH_2-CH_3). **Elemental analysis** for $C_{18}H_{19}FN_4OS$: calculated (%) C 60.32, H 5.34, F 5.30, N 15.63, O 4.46, S 8.95; found: C 60.38, H 5.39, F 5.20, N 15.69, O 4.40, S 8.90.

N-(allyl)2-[2-(3-fluoro-9H-carbazol-9-yl) acetyl]-hydrazine-1-carbothioamide (4q)

Appearance: White solid isolated; Mp: 205–207 °C; Yield: 78%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.31 (s, 1H), 9.52 (d, J = 93.9 Hz, 1H), 8.20–8.11 (m, 2H), 7.64–7.47 (m, 2H), 7.44 (ddd, J = 8.3, 7.1, 1.3 Hz, 2H), 7.30–7.14 (m, 2H), 5.86 (ddt, J = 17.3, 10.2, 5.0 Hz, 1H), 5.25–4.98 (m, 4H), 4.17 (d, J = 5.6 Hz, 2H). ^{13}C NMR (100 MHz, DMSO) δ: 170.98 (C=S), 167.73 (C=O), 141.14, 141.06 (C-3, C–F), 135.31, 126.11, 126.02, 122.71, 120.64, 119.55, 119.45 (Ar–CH), 116.25, 115.75, 109.92, 109.76 (carbazole-CH), 46.34, 44.44 (N-CH_2). **Elemental analysis** for $C_{18}H_{17}FN_4OS$: calculated (%) C 60.66, H 4.81, F 5.33, N 15.72, O 4.49, S 9.00; found: C 60.60, H 4.78, F 5.35, N 15.62, O 4.60, S 9.05.

N-(propyl)2-[2-(3-fluoro-9H-carbazol-9-yl) acetyl]-hydrazine-1-carbothioamide (4r)

Appearance: White solid isolated; Mp: 173 –175 °C; Yield: 84%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.22 (s, 1H), 9.41 (d, J = 104.2 Hz, 1H), 8.16 (d, J = 7.7 Hz, 2H), 8.01 (d, J = 5.6 Hz, 1H), 7.56 (d, J = 8.2 Hz, 2H), 7.46 (t, J = 7.5 Hz, 3H), 7.23 (dd, J = 8.8, 6.2 Hz, 2H), 5.11 (s, 2H), 2.95 (d, J = 4.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO) δ:

171.43 (C=S), 167.79 (C=O), 141.04 (C-3, C–F), 126.14, 126.03, 122.68, 120.67, 120.62, 119.56, 119.44 (Ar–CH), 109.88, 109.76 (carbazole-CH), 44.39, 31.42 (N-CH_2). **Elemental analysis** (%) for $C_{18}H_{19}FN_4OS$: calcd: C 60.32, H 5.34, F 5.30, N 15.63, O 4.46, S 8.95; found: C 60.25, H 5.40, F 5.50, N 15.53, O 4.36, S 8.85.

All the synthesised derivatives **4(a-r)** were confirmed by ^{1}H NMR,^{13}C NMR, and HRMS/elemental analysis as described above. The chromatograms and NMR spectra are available in the Supporting Information.

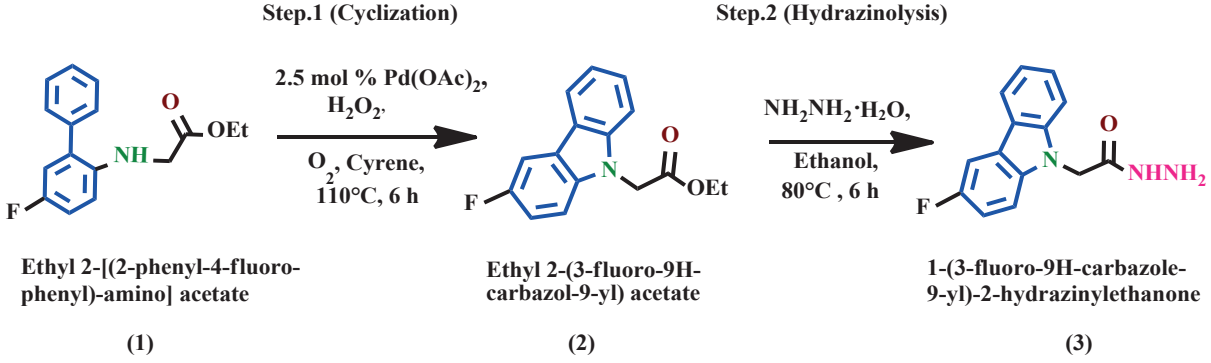
RESULTS AND DISCUSSION

The synthesis of derivatives (2) and (3) are presented in (Scheme 1). In step 1, the cyclization reaction (Scheme 1), ethyl 2-[(2-phenyl-4-fluorophenyl) amino] acetate (1) was reacted in cyrene in the presence of Pd $(OAc)_2$ and H_2O_2 at an appropriate temperature with continuous bubbling of air. The solid was isolated by precipitation, dried, and recrystallized from isopropyl alcohol to obtain pure ethyl 2-(3-fluoro-9H-carbazol-9-yl) acetate (2) with an excellent yield of 93%. The analytical and spectroscopic data for compound (2) are summarized below. The analysis of the column chromatography with a retardation factor (R_f) of 0.32 in the EtOAc: DCM (9:1) solvent system and the melting point (M.p) of 245–247 °C, is supported by the literature value in the range of 245–248 °C. The structure of ethyl 2-(3-fluoro-9H-carbazol-9-yl) acetate (2) was confirmed by ^{1}H NMR, ^{13}C NMR spectroscopy, and elemental analysis. In the ^{1}H NMR spectrum (DMSO-d_6), the aromatic carbazole protons appeared in the region δ 8.17–7.24 ppm, with characteristic signals at δ 8.17 (d, *J* = 3.1 Hz, 2H), 7.56 (dd, *J* = 7.9, 3.1 Hz, 2H), 7.51–7.43 (m, 2H), and 7.24 (d, *J* = 3.0 Hz, 2H), which were consistent with the substituted carbazole framework. The singlet at δ 5.35 ppm (2H) was assigned to the N–CH_2 group linking the carbazole nucleus to the acetate moiety. The ethyl ester functionality was evidenced by signals at δ 4.23–4.11 ppm (m, 2H, O–CH_2) and 1.22 ppm (t, 3H, CH_3). The ^{13}C NMR spectrum further supported the proposed structure, displaying resonances at δ=167.48 ppm for the ester carbonyl carbon and δ=165.94 ppm for the fluorinated aromatic carbon (C–F). Signals corresponding to the carbazole ring carbons were observed between δ 141.06 and 109.99 ppm, while the methylene carbon attached to nitrogen appeared at δ 44.59 ppm, and the ethoxy carbons were observed at δ 40.62–40.20 and 39.99–39.37 ppm. Elemental analysis showed excellent agreement between the calculated and experimental values for $C_{16}H_{14}FNO_2$ (calcd: C, 70.84; H, 5.20; F, 7.00; N, 5.16; O, 11.80%; found: C, 70.63; H, 5.25; F, 7.10; N, 5.25; O, 11.86%), confirming the purity and molecular composition of compound (2). Collectively, the spectroscopic and analytical data support the assigned structure of ethyl 2-(3-fluoro-9H-carbazol-9-yl) acetate.

The synthesised 2-(3-fluoro-9H-carbazol-9-yl) acetohydrazide (3) was obtained via hydrazinolysis as an off-white crystalline solid in 81% yield after recrystallization from isopropyl alcohol. The compound exhibited a sharp melting point of 239–241 °C, indicating high purity. Structural elucidation was accomplished using ^{1}H and ^{13}C NMR spectroscopy and elemental analysis. The ^{1}H NMR spectrum (DMSO-d_6) displayed a characteristic singlet at δ 9.54 ppm corresponding to the hydrazide NH proton, confirming the successful conversion of the ester precursor into the corresponding hydrazide derivative. The aromatic protons of the fluoro-carbazole framework resonated between 8.20 and 7.22 ppm, appearing as multiplets and doublets, consistent with the substitution pattern of the carbazole nucleus. A singlet at δ 4.99 ppm, integrating for two protons, was assigned to the methylene group attached to the carbazole nitrogen (N–CH_2–CO), while the signal at δ 4.33 ppm (d, J = 4.3 Hz, 2H) was attributed to the hydrazide NH_2 protons, further supporting the formation of the acetohydrazide functionality. The ^{13}C NMR spectrum shows a prominent resonance at δ 167.23 ppm, corresponding to the amide carbonyl carbon (C=O). The aromatic carbons of the fluoro carbazole scaffold appeared within the expected range of δ 141.04–109.91 ppm, including the fluorine-bearing aromatic carbon at δ 119.45 ppm. The methylene carbon linking the carbazole nitrogen to the carbonyl group resonated at δ = 44.67 ppm. These spectral features are in excellent agreement with the proposed structure of compound (3). The molecular composition was further confirmed by elemental analysis. The experimentally determined values (% w/w) (C: 65.45; H: 4.55; F: 7.30; N: 16.30) closely matched the calculated values for $C_{14}H_{12}FN_3O$ (C: 65.36; H: 4.70; F: 7.38; N: 16.33; O: 6.22), demonstrating the high purity and correct molecular formula of the synthesised product. Overall, the spectroscopic and analytical data confirmed the structure of 2-(3-fluoro-9H-carbazol-9-yl) acetohydrazide (3).

In step 3 (Scheme 2), for the rearrangement, 1-(3-fluoro-9H-carbazole-9-yl)-2-hydrazinylethanone (4.18 mmol, 1000 mg) (3) and 4-chlorophenyl iso thiocyanates (3.28 mmol, 1000 mg) was combined at 80°C in 10 mL ethanol to determine the ideal conditions for the synthesis of 2-(2-(3-fluoro-9H-carbazol-9-yl)acetyl)-N-(4-chlorophenyl)hydrazine-1-carbothioamide (900 mg, 90% yield) **(4a).**

This was followed by comparison of the ultrasonic and traditional methods as described in Table 1. The reaction was first attempted at low (50°C) and then high (70°C) temperature using both approaches without the use of a solvent (entries 1 and 2), with no product initiated. Additionally, optimisation showed that since toluene is a nonpolar solvent, it can be used to initiate the transformation thus obtaining a yield of 50% (30 min, ultrasonic method) instead of 38% using the conventional approach (entry 3). Interestingly, when the reaction was done under the THF solvent conditions (entry 4), the yield improved to 72% (ultrasonic method 30 min) compared to 56% (conventional method, 2h). When using DMF as the solvent (entry 5), the yield obtained was 76% (ultrasonic method, 30 min) compared to 58% (conventional method, 2h), suggesting that the moderate polar to polar solvent conditions may enhance the yield. The next reaction was carried out in water (entry 6), and the achieved yield was 60% (ultrasonic method, 30 min) and 42% (conventional method, 2h). In this reaction (entry 6), the yield is reduced (conventional method) possibly due to the less soluble starting materials in water. The best

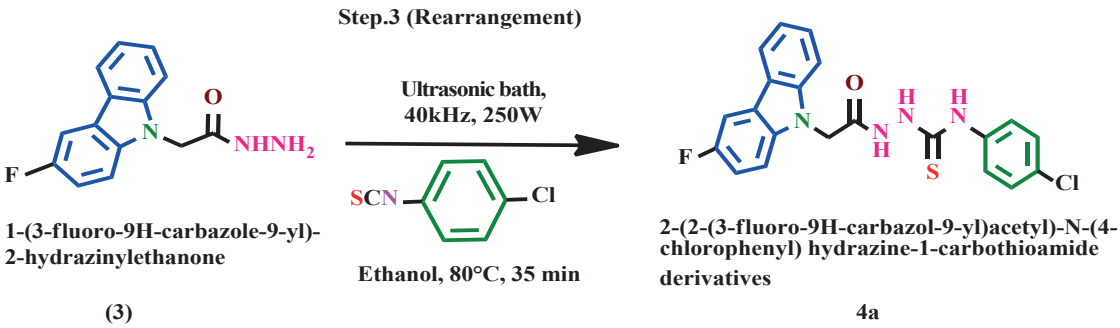


Ethyl 2-[(2-phenyl-4-fluoro-phenyl)-amino] acetate **(1)**

Ethyl 2-(3-fluoro-9H-carbazol-9-yl) acetate **(2)**

1-(3-fluoro-9H-carbazole-9-yl)-2-hydrazinylethanone **(3)**

Scheme 1. Synthesis of 1-(3-fluoro-9H-carbazole-9-yl)-2-hydrazinylethanone (3)



Scheme 2. Synthesis of 2-(2-(3-fluoro-9H-carbazol-9-yl) acetyl)-N-(4-chlorophenyl) hydrazine-1-carbothioamide **(4a)**.

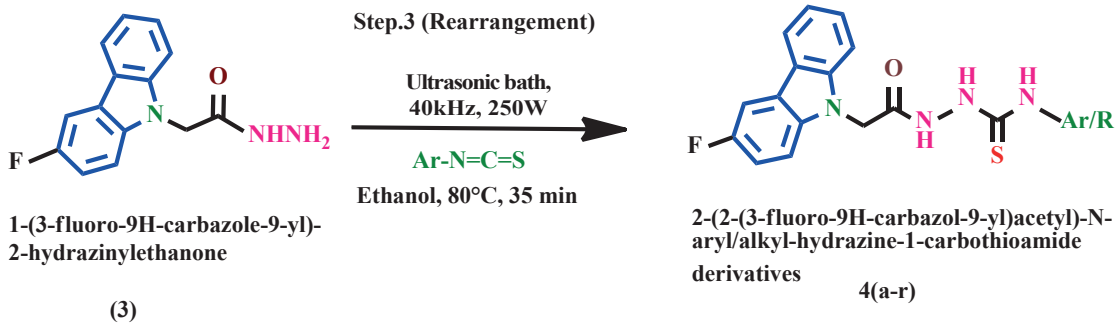
Table 1. Optimisation of the reaction conditions for model compounda (**4a**)

Entry	Ultrasonication method		Conventional method	
	Experimental conditions: ultrasonic bath, 40kHz, 250W Solvent, temp., reaction time	%Yield	Experimental conditions: conventional oil bath Solvent, temp., reaction time	%Yield
1	Solvent-free, 50°C, 25 min	Trace	Solvent-free, 80°C, 1h	Trace
2	Solvent-free, 70°C, 15 min	Trace	Solvent-free, 100°C, 1h	Trace
3	Toluene, 80°C, 30 min	52	Toluene, 80°C, 2h	38
4	THF, 65°C, 35 min	72	THF, 65°C, 2h	56
5	DMF, 80°C, 30 min	76	DMF, 110°C, 2h	58
6	water, 80°C, 30 min	60	water, 80°C, 2h	42
7	Methanol, 65°C, 30 min	84	Methanol, 65°C, 2h	66
8	Ethanol, 80°C, 35 min	92	Ethanol, 80°C, 2h	74
9	Isopropyl alcohol, 80°C, 30 min	86	Isopropyl alcohol, 80°C, 2h	70
10	n-propyl alcohol, 85°C, 40 min	72	n-propyl alcohol, 85°C, 2h	65

[a]The progress of the reaction was monitored by TLC and HPLC methods; 1-(3-fluoro-9H-carbazole-9-yl)-2-hydrazinylethanone (1.0 equiv.), 4-chlorophenyl-isothiocyanates (1.0 equiv.), and solvent (10 mL)

results were obtained when the reaction was done in polar protic solvents: methanol (entry 7), ethanol (entry 8), and isopropyl alcohol (entry 9), with good to excellent yields achieved. The best optimised conditions (92% yield) were with the preferred solvent ethanol (ultrasonic method, 35 min). Ironically, this was also the best yield (74%) for the conventional method. These results proved that the polar protic solvent was the best for the derivatives as they stabilized the intermediates through hydrogen bonding and dipole-dipole interactions, lowering the activation energy of the rate-determining step. When the reaction was carried out using n-propyl alcohol (entry 10), the yield decreased to 72% (ultrasonic method, 40 min) compared to 65% (conventional method, 2h). This may be due to the structure of the alcohols having the hydroxyl group on a terminal carbon, which dictates the reactivity and makes them less prone to transformation, compared to secondary and primary alcohols.

Based on the results presented in Table 1, ultrasonication outperforms the conventional method in terms of yield, temperature, reaction time and solvent used for both methods. These findings demonstrate the importance of ultrasonic irradiation in accelerating the synthesis of 3-fluoro-carbazole derivatives. Using the optimised reaction conditions, 3-fluoro carbazole linked acetyl hydrazine-1-carbothioamide derivatives 4(a-r) were synthesised by reacting compound (3) with different substituted isothiocyanates in an ultrasonic bath (40 kHz, 250W) with 10 mL of ethanol at 80°C (Scheme 3). The reaction was monitored using the TLC and was completed in 35-40 minutes (ultrasonication) and in 2-3 hours using the conventional method. The final product was collected by removing of the solvent under vacuum, and purifying the resulting solid using column chromatography to get an excellent yield. The melting point, yield and reaction time of the resulting compounds were checked by comparing this reaction step with the traditional approach (Table 2). Column chromatography was used to purify all the produced compounds, and HPLC, ^{1}H NMR, ^{13}C NMR, and mass spectra were obtained in order to evaluate the structure.



Scheme 3. Synthesis of new N-substituted-2-[2-(3-fluoro-9H-carbazol-9-yl) acetyl] hydrazine-1-carbothioamide derivatives **4(a-r).**

Table 2. N-substituted-2-[2-(3-fluoro-9H-carbazol-9-yl) acetyl] hydrazine-1-carbothioamide derivatives **4(a-r)** produced under ultrasonication vs conventional method

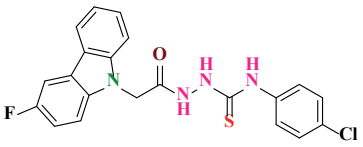
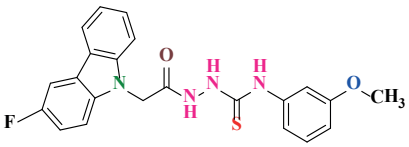
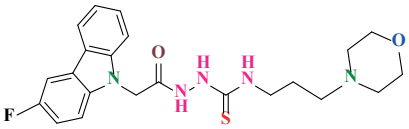
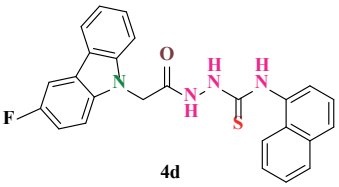
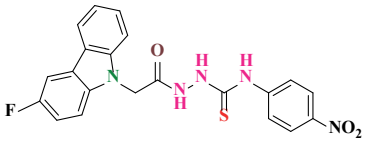
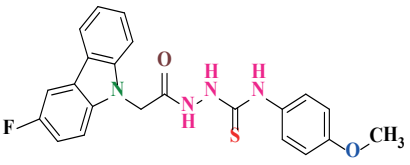
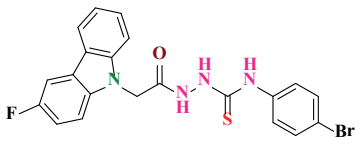
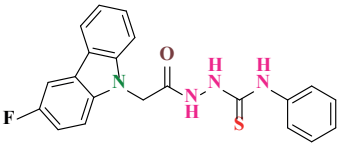
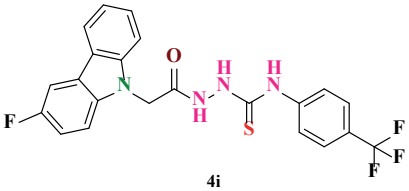
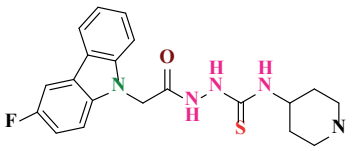
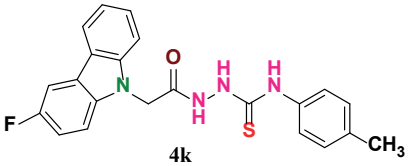
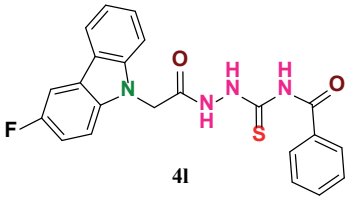
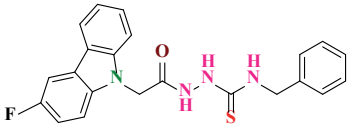
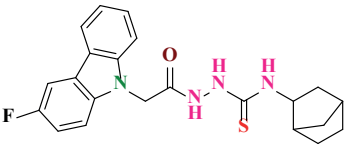
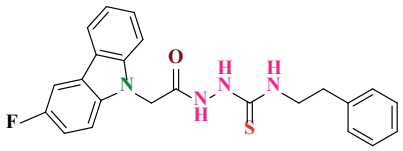
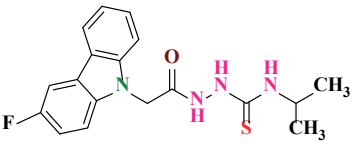
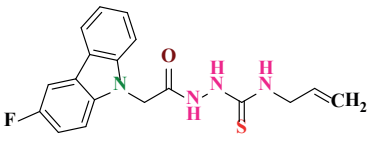
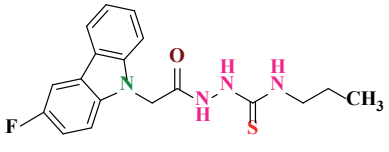
Compounds	M.p °C (observed)	Ultrasonication method		Conventional method	
		% Yield	Time[a] (min)	% Yield	Time[a] (hour)
 **4a**	235–237	92	35	74	2
 **4b**	218–220	90	32	68	2.5
 **4c**	189-191	88	30	65	2
 **4d**	222–224	90	40	70	3
 **4e**	235-237	86	38	70	2.5
 **4f**	210–212	90	30	72	2
 **4g**	255–257	88	32	74	2.5
 **4h**	235-237	90	30	76	2
 **4i**	277-279	92	35	78	3

Table 2: (cont.)

Compounds	M.p °C (observed)	Ultrasonication method		Conventional method	
		% Yield	Time[a] (min)	% Yield	Time[a] (hour)
 **4j**	211-213	88	30	71	2.5
 **4k**	180–182	90	30	71	2
 **4l**	189-191	89	30	66	2
 **4m**	169-171	88	30	68	2
 **4n**	199-201	85	40	70	3
 **4o**	170–172	86	40	72	3
 **4p**	186-188	90	30	70	2
 **4q**	205-207	92	35	76	2
 **4r**	173-175	92	35	75	2

[a]Reaction time.

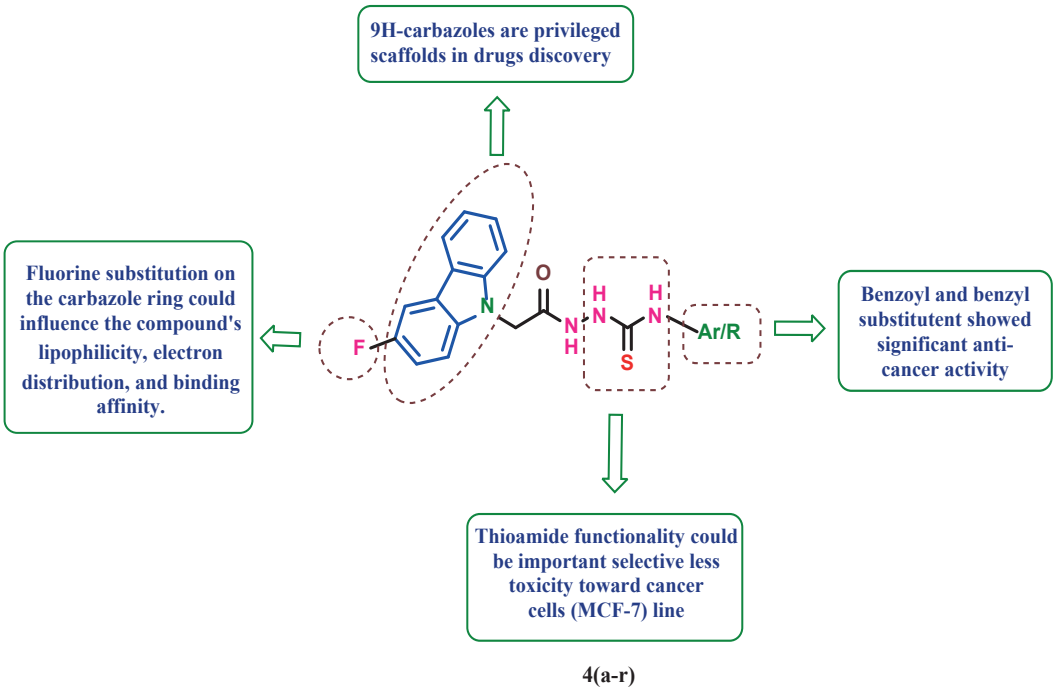


Figure 2. Structure–activity relationship (SAR) of N-substituted-2-[2-(3-fluoro-9H-carbazol-9-yl) acetyl] hydrazine-1-carbothioamide derivatives **4(a-r)**

Biological Activity

Despite the development and manufacture of many anticancer drugs for chemotherapy, a therapeutic product with an acceptable level has not yet been identified. There is still a need for cancer treatment, as some drugs have demonstrated resistance and adverse effects to several cancers. Nevertheless, efforts are being made to develop novel anticancer drugs. In this study the 18 novel compounds were examined for their cytotoxic and antiproliferative properties (Figure 2). The 18 new compounds were tested against human breast cancer (MCF-7) cell lines to determine their antiproliferative activity (Figure 2).

The screening of the IC_{50} values of the synthesised derivatives **4(a-r)** is presented in Table 3. IC_{50} values greater than 100 µM were considered inactive. The results, expressed as IC_{50} values (the concentration required to inhibit 50% of cell growth), revealed a broad spectrum of activity ranging from highly potent to non-inhibitory (NI). The IC50 result for the reference, Etoposide, was 10.52 µM.

The variation of the IC_{50} values across the synthesised derivatives, highlights the significant impact of the R/Ar functional groups on the aromatic ring as well as the fluorine atom on the carbazole scaffold. Highly potent derivatives include the benzoyl (**4l**, 9.00 µM), benzyl (**4m**, 9.08 µM), trifluoromethyl (**4i,** 10.02 µM**),** and phenyl ethyl (**4o**, 10.90 µM) due to the excellent characteristics of the functional group, which includes membrane permeability, binding pocket affinity, and structural flexibility. Also, the strong electron-withdrawing nature and binding specificity of the trifluoromethyl groups are important. Furthermore, the moderate potency of 3-methoxy **(4f**, 12.02 µM), p-chlorophenyl (**4a**, 12.05 µM), nitro (**4e**, 13.05 µM), bromo (**4g**, 14.04 µM), 4-methoxy (**4b**, 15.02 µM), and methyl (**4k**, 16.04 µM) may be attributed to the halogen and methyl substituents, which possibly enhance hydrophobic interactions and membrane permeability. The methoxy and nitro groups may contribute weak hydrogen-bonding or dipolar interactions. However, none of these substituents provide sufficiently strong or highly specific binding interactions to significantly enhance target affinity, resulting in only moderate cytotoxic activity. Low potency was observed for morpholyl (**4c**, 18.04 µM), isopropyl (**4p**, 20.01 µM), allyl (**4q**, 21.08 µM), phenyl (**4h**, 22.00 µM), and isopropyl (**4r**, 23.01 µM). A possible explanation for this is may be the substituents disturb the balance between lipophilicity, electronic effects, steric compatibility, and hydrogen bonding capacity, leading to weak target binding thus lowering the antiproliferative activity. Compounds such as naphthalene (**4d**), piperidine (**4j**), and norbornyl (**4n**) exhibited IC50 values that are >50 µM and were inactive due to the bulky, highly hydrophobic naphthalene group which causes steric hindrance within the binding pocket resulting in reduced aqueous solubility and limited effective cellular uptake. Although piperidine is basic and potentially hydrogen-bonding, it introduces increased polarity and possible protonation at physiological pH, which may alter membrane permeability and disrupt optimal binding interactions of the drug. The norbornyl group is rigid and bulky, creating significant steric strain which prevents alignment of the pharmacophore within the active site. These results demonstrate the diverse range of antiproliferative properties of the novel compounds and highlight the need for additional study and evaluation to understand their potential in comparison to other well-known antiproliferative medications.

CONCLUSION

Using commercial ethyl 2-[(2-phenyl-4-fluoro-phenyl)-amino] acetate and facilitated by ultrasonication, a straightforward, green synthetic method was developed to access an array of 3-fluoro-9H-carbazoles (85-92% yield) with a palladium acetate catalyst and cyrene as a green solvent. This study evaluated the anticancer activity of compounds with a 3-fluoro-9H-carbazole framework hybridized with a thiosemicarbazide functional group. The synthesised derivatives of N-substituted-2-[2-(3-fluoro-9H-carbazol-9-yl) acetyl] hydrazine-1-carbothioamide derivatives **4(a-r)** displayed good antiproliferative activity against breast cancer. Compounds **4l** (9.00 µM) and **4m** (9.08 µM) with the benzoyl and benzyl substituents showed enhanced antiproliferative activity compared to the reference drug, Etoposide (10.52 µM). Compounds **4l** and **4m** also demonstrated potential anticancer properties. These compounds altered the pathway, causing apoptosis and halting the cell cycle in MCF-7 cancer cells. Targeting this pathway provides a promising method for lowering proliferation survival and combatting treatment resistance. This study contributes significantly to our understanding of the biological effects of 3-fluoro-9H-carbazole derivatives and sheds light on the development of more effective anticancer treatment possibilities. However, additional in vivo and in vitro experiments are required to validate and optimise possible therapeutic drugs for future clinical uses.

Table 3. Antiproliferative activity of new N-substituted-2-[2-(3-fluoro-9H-carbazol-9-yl) acetyl] hydrazine-1-carbothioamide derivatives **4(a-r)** against human breast cancer (MCF-7) cell lines[a]

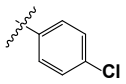
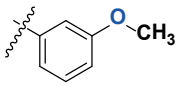
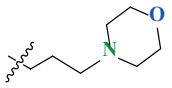
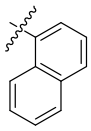
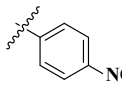
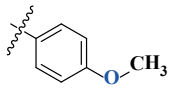
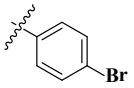
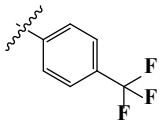
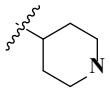
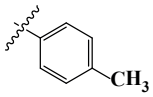
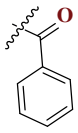
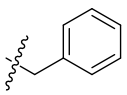
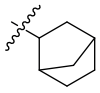
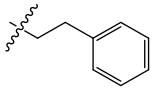
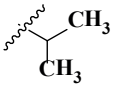
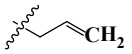
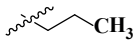
Compound Code	R/Ar	(IC_{50} ± SD, µM) values[b] Carbazole Derivatives Effect on MCF-7 Cell Line[a]
4a		12.05 ± 0.04 µM
4b		15.02 ± 0.05 µM
4c		18.04 ± 0.08 µM
4d		NI
4e		13.05 ± 0.05 µM
4f		12.02 ± 0.02 µM
4g		14.04 ± 0.02 µM
4h		22.00 ± 0.01 µM
4i		10.02 ± 0.0 µM
4j		NI
4k		16.04 ± 0.04 µM
4l		9.00 ± 0.02 µM
4m		9.08 ± 0.05 µM
4n		NI
4o		10.90 ± 0.40 µM

Table 3: (cont.)

Compound Code	R/Ar	(IC_{50} ± SD, µM) values[b] Carbazole Derivatives Effect on MCF-7 Cell Line[a]
4p		20.01 ± 0.02 µM
4q		21.08 ± 0.06 µM
4r		23.01 ± 0.04 µM
Etoposide[c]	–	10.52 ± 0.20 µM[a]

[a]Cell lines: human breast cancer (MCF-7) cell lines; [b]Results are expressed as mean values ± SD; [c]Etoposide (reference), IC_{50} values of the compounds that are higher than 100 µM considered as no inhibition (NI)

SUPPLEMENTARY INFORMATION

The Supplementary Information for this article is provided in a separate document. supplement.

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AUTHOR CONTRIBUTIONS

Writing original draft; data curation; software; formal analysis and interpretation of results – B.S.H; Conceptualization, writing, review and editing – S.B.G.

DECLARATION OF COMPETING/FINANCIAL INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES

The authors declare that no generative AI or AI-assisted technologies were used in the preparation of this manuscript.

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