Synthesis of Anthracene Functionalised Fluorescent Amino Acids and Its Applications of Biosensing and Antibacterial Activity

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ABSTRACT: Anthracene based fluorescent amino acid were synthesized and characterized by spectral methods. The fluorescence marking of MCF7 cell lines with these amino acids were discussed. Some of the selected anthracene compounds shown antimicrobial activities against \textit{Micrococcus luteus}, \textit{Staphylococcus aureus}, \textit{Bacillus cereus}, \textit{Bacillus subtilis}, \textit{Candida albicans}, \textit{Escherichia coli}, \textit{Pseudomonas aeruginosa}, \textit{Proteus vulgaris} and \textit{Shigella flexneri}. Among the organism tested, maximum growth inhibition was observed with \textit{Micrococcus luteus}, \textit{Candida albicans}.

KEYWORDS: Amino acid, peptide, fluorescence, biosensing, antibacterial activity.

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I. INTRODUCTION

Fluorescence mediated bio-imaging is an excellent analytical tool for labeling cells in-vitro and in-vivo studies. These probes are valuable for the investigation of biochemical processes, diagnosis of disease markers, detection of hazardous compounds, and other purposes. Further it is very sensitive, fast-responding, and is capable of affording high spatial resolution via microscopic imaging.

Several fluorescent probes have been reported to investigate biological process through fluorescence measurements\textsuperscript{1}, such as carbohydrate based detection of bacterial pathogens\textsuperscript{2} in food industry, water and environment quality control, and clinical diagnosis. Fluorescein has been used as a platform for many kinds of fluorescent probes\textsuperscript{3}. 

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Synthesis of anthracene functionalised fluorescent amino acids and its applications of biosensing and antibacterial activity.}
\end{figure}
Green fluorescent protein⁴ from jellyfish *Aequorea victoria* is also most extensively studied and widely used in cell biology protein such as monitoring of gene expression profiles⁵ and protein localizations⁶ in a variety of systems.

Various kinds of fluorescent derivatives have been reported for bioimaging such as synthetic receptors⁷, proteins⁸, aptamers⁹, quantum dots (QDs)¹⁰, BODIPY derivatives¹¹, quinoline derivatives¹² for bacterial detection, pH fluorescence probes¹³ and so forth¹⁴-¹⁶.

Eventhough various organic fluorophores have been well-established for labeling of biomolecules in various diagnostic applications, but they have limited effectiveness in long-term imaging and multiplexed detection due to their inherent demerits¹⁷. Generally, the molecular fluorescent probe consists of a sensing moiety (auxochrome) as the key component for generating the fluorescence signal and an analyte-responsive receptor (recognition moiety), both of which are linked by a spacer. Though there are many fluorescent chromophores reported and commercially available, there is a continuous need for new fluorescent molecules owing to their photo instability. We intend to design a new methodology for making fluorescent amino acids wherein a desirable amino acid residue can be easily included with the fluorescent tag.

Thus we have designed a series of fluorescent sensors¹⁸ for bioimaging¹⁹ by linking a fluorophore unit, anthracene, and an amino acid subunit, like glycine, alanine, L-tyrosine, DL-serine through phenyl ring (Figure-1). The amino acid unit can fluorescently label the cells, which can show fluorescence thereby providing a sensitive detection method. The main objective of the present investigation was to label the cancer cells and will facilitate fluorescent imaging of cells.

**Amino acid as fluorescent label**

![Anthracene based amino acid as fluorescent label](image)

**Figure 1** Anthracene based amino acid as fluorescent label

## II. RESULTS AND DISCUSSION

Symyx technologies²⁰ group developed the synthesis of 3-(anthracen-10-yl)-2-(methoxymethoxy)-5-methylbenzaldehyde under different conditions. After a thorough review of all the conditions, we have modified and reported a new synthetic scheme for the synthesis of MOM protected anthracenyl aldehyde with simple purification techniques.
Scheme 1 Synthesis of fluorescent amino acids and hydroxyl amino acids

The protection of phenolic group in 2-bromo-4-methyl phenol (2) was carried with methoxy methyl chloride, diisopropyl ethyl amine and dichloromethane at room temperature over a period of 4 h to afford intermediate 3 with good yield. Suzuki coupling was carriedout with 3 and anthracenoboronic acid usingsodium carbonate, and tetrakis(triphenylphosphine)palladium in toluene at 110 °C for 8 h to afford 4. Same suzuki reaction was also attempted by taking reverse analogues, 9-bromo anthracene and 2-(methoxymethoxy)-5-methylphenylboronic acid with the same condition, there observed very less yield. Further methoxy methyl cleavage was carried out by treating 4 with 4.5 M HCl in dioxane at 0 °C to room temperature for 5 h to afford 2-anthracenyl phenolic intermediate (5).Bromination of 5 was attempted by treatment with N-bromosuccnimide, triethyl amine and dichloromethane as solvent at room temperature for 8 h to afford (6) anthracenylbromo phenol. The same step was tried with bromine in chloroform or bromine acetic acid at 0 °C to room temperature, the reaction proceeds with many spots in TLC. Compound 6 was protected with MOMCl using Hunig’s base and DCM for 4 h to get analytically pure MOM protected bromo compound 7 after column purification. Formylation of 7 was attempted by using n-BuLi and DMF at -78°C to afford 3-(anthracen-10-yl)-2-(methoxymethoxy)-5-methylbenzaldehyde (8) as yellow solid.

Reductive amination reaction was carried out with 8 and different amino acids (glycine, alanine, L-tyrosine, DL-serine) using sodium borohydride solution in ethanol under nitrogen atmosphere for 8 h to afford crude fluorescent amino esters, which was hydrolysed by using 4 equiv of lithium hydroxide and mixture of solvents (tetrahydrofuran: methanol: water) at room temperature for 15 h to get 9 (a-d).

The MOM cleavage of 9 (a-d) was carried out using 4.5 M HCl in dioxane at room temperature for 5 h to afford 10 (a-d). All the titled compounds were characterized by 1H NMR, 13C NMR, LCMS and HPLC.
Synthesis and characterisation

Synthesis of 2-bromo-1-(methoxymethoxy)-4-methylbenzene (3)

To a solution of 2-bromo-4-methyl phenol (2) (7.5 g, 40.53 mmol) and diisopropyl ethyl amine (20.14 mL, 121.5 mmol) in 75 mL of dichloromethane, methoxymethyl chloride (12.4 mL, 162 mmol) was added dropwise at 0 °C for 30 min and the solution was stirred at room temperature for 4 h. The reaction was monitored by TLC, after completion, the reaction was quenched by the addition of water (40 mL), extracted with DCM (3×20 mL), organic layer was washed with water (80 mL), brine (50 mL), and dried over sodium sulphate and concentrated to afford crude product 2-bromo-1-(methoxymethoxy)-4-methylbenzene (3) (8.9 g, yield 95.02 %) was taken as such for the next step without purification as a yellowish oil. 

\[^{1}H\text{ NMR (CDCl}_3/300\text{ MHz); \delta 7.26 (1H, s, H}_2) \text{, 7.08 -7.05 (2H, m, H}_4, \text{H}_5), 5.26 (2H, s, H}_8), 2.97 (3H, s, H}_9), 2.27 (s, 3H, H}_0) \text{ ppm.}\]

\[^{13}C\text{ NMR (CDCl}_3/75\text{ MHz); \delta 151.3 (C}_6, 134.5 (C}_2, 132.9 (C}_3, 129.1(C}_4), 116.4(C}_5), 112.1(C}_1), 94.3(C}_8), 55.3(C}_9), 23.1(C}_7) \text{ ppm.}\]
Synthesis of 9-(2-(methoxymethoxy)-5-methylphenyl) anthracene (4)

A mixture of 2-bromo-1-(methoxymethoxy)-4-methylbenzene (3) (8 g, 34.6 mmol) and anthracene-9-boronic acid (9.9 g, 45 mmol) in toluene (40 mL) was stirred for 10 min at room temperature. To this mixture, nitrogen purged aqueous sodium carbonate solution (14.5 g in 40 mL) was added followed by tetrakis(triphenylphosphine)palladium(0) (1.9 g, 1.7 mmol). The reaction mixture was heated at 110 °C for 5-8 hrs. After completion, the reaction mixture was cooled to room temperature, diluted with ethyl acetate and filtered through celite bed. The filtrate was washed with water (2x80 mL) and brine solution (100 mL). The organic layer was separated, dried over anhydrous sodium sulfate, and filtered. The filtrate was evaporated to dryness under vacuo. The residue was purified by using column chromatography (silica gel (60-120 mesh), 40 % ethyl acetate in n-hexane) to offer analytically pure product (4) 9-(2-(methoxymethoxy)-5-methylphenyl) anthracene (101 mg, 86 %) as white solid. NMR (CDCl₃/400 MHz); δ 8.49 (1H, s, H₁₃), 8.06-8.03 (2H, d, J=8.43 Hz, H₁₉, H₂₀), 7.68-7.65 (2H, d, J=8.43, H₁₆, H₂₁), 7.48-7.43 (2H, m, H₁₈, H₂₂), 7.38-7.1 (4H, m, H₄, H₅, H₁₇, H₂₂), 7.10 (1H, m, H₂), 4.90 (2H, s, H₈), 3.21 (3H, s, H₉), 2.39 (3H, s, H₇) ppm. LCMS m/z: 285.4 (M⁺+1).
Synthesis of 2-(anthracen-10-yl)-4-methylphenol (5)

To a solution of 9-(2-(methoxymethoxy)-5-methylphenyl)anthracene (4) (8 g, 24.3 mmol) in 1,4-dioxane (80 mL) , 4.5 M HCl in dioxane (50 mL) was added dropwise at 0 °C, the resulting mixture was stirred at room temperature for 5 h. The reaction was monitored by TLC; after completion, the reaction mixture was quenched by the addition of water (30 mL), compound was extract with ethyl acetate (3×20 mL), organic layer was washed with water (50 mL), brine solution (2×30 mL), and dried over anhydrous Na2SO4. The solvent was evaporated under reduced pressure to yield the crude product which was purified by column chromatography (silica gel (60-120 mesh), eluted with 60 % ethyl acetate in n-hexane) to afford 2-(anthracen-10-yl)-4-methylphenol (5) (6.5 g, 93.8 %) as white solid. \(^1\)H NMR (CDCl₃/400 MHz); δ 8.57 (1H, s, H₁₃), 8.09-8.07 (2H, m, H₁₉, H₂₀), 7.72-7.69 (2H, m, H₁₆, H₂₃), 7.52-7.48 (2H, m, H₁₈, H₂₁), 7.44-7.40 (2H, m, H₁₇, H₂₂), 7.29 (1H, m, H₂), 7.08-7.06 (2H, m, H₄, H₅), 4.37 (1H, s, OH), 2.38 (3H, s, H₇) ppm. LCMS m/z: 285.4 (M⁺+1).
Synthesis of 2-(anthracen-10-yl)-6-bromo-4-methylphenol (6)

To a solution of 2-(anthracen-10-yl)-4-methylphenol (5) (5 g, 17.5 mmol) and diisopropylamine (6.2 mL, 43.9 mmol) in DCM (100 mL) was added N-bromosuccinimide (3.4 g, 19.3 mmol) solution in DCM (150 mL) dropwise, the resulting mixture was stirred at room temperature for 8 h. The reaction was monitored by TLC; after completion, the reaction mixture was quenched by the addition of water (70 mL) and the compound was extracted with DCM (3x20 mL), washed with water (2x70 mL) and saturated brine (50 mL), dried the organic layer over anhydrous Na$_2$SO$_4$. Evaporation of the solvent under vacuum furnished the crude product. It was crystallized by dissolving in ethyl acetate and triturate with n-hexane till the solid precipitated out which was filtered and dried to afford 2-(anthracen-10-yl)-6-bromo-4-methylphenol (6) (5.2 g, 81.4 %) as off-white solid. $^1$H NMR (CDCl$_3$/ 300 MHz); δ 8.56 (1H, s, H$_{13}$), 8.09-8.06 (2H, d, $J$=8.37 Hz, H$_{19}$, H$_{20}$), 7.66-7.53 (2H, d, $J$=8.76 Hz, H$_{16}$, H$_{23}$), 7.53-7.27 (5H, m, H$_2$, H$_{17}$, H$_{18}$, H$_{21}$, H$_{22}$), 7.05 (1H, s, H$_4$), 5.08 (1H, s, OH), 2.37 (3H, s, H$_7$) ppm. LCMS m/z: 361.1 (M$^+$-2).
Synthesis of 10-(3-bromo-2-(methoxymethoxy)-5-methylphenyl) anthracene (7)

To a solution of 2-(anthracen-10-yl)-6-bromo-4-methylphenol (6) (2 g, 5.5 mmol) and diisopropyl ethyl amine (2.73 mL, 16.5 mmol) in dichloromethane (20 mL) was added methoxy methyl chloride (1.3 mL, 16.5 mmol) dropwise at 0°C over 30 min and the solution was stirred at room temperature for 4h. The reaction was monitored by TLC, after completion, the reaction was quenched by the addition of water (10 mL) and then the reaction mixture was extracted with DCM (2x10 mL), washed with water (20 mL), saturated brine (10 mL) and dried the organic layer over anhydrous Na$_2$SO$_4$ and concentrated under reduced pressure and the crude product was subjected to column
chromatography (silica gel (60-120 mesh), 60% ethyl acetate in n-hexane as eluent) to afford (7) 10-(3-bromo-2-(methoxymethoxy)-5-methylphenyl)anthracene (1.9 g, 84.8 %) as off-white solid. \( ^1H \) NMR (CDCl\(_3\)/ 300 MHz); \( \delta \) 8.51 (1H, s, H\(_{13}\)), 8.05-8.02 (2H, d, \( J=8.13 \) Hz, H\(_{19}, H_{20}\)), 7.68-7.60 (3H, m, H\(_2, H_{16}, H_{23}\)), 7.50-7.38 (4H, m, H\(_{17}, H_{18}, H_{21}, H_{22}\)), 7.11-7.1 (1H, d, H\(_4\)), 4.47 (2H, s, H\(_8\)), 2.39 (3H, s, H\(_9\)), 2.30 (3H, s, H\(_7\)) ppm. LCMS \( m/z \): 409.1 (M\(^+2\)).

**Synthesis of 3-(anthracen-10-yl)-2-(methoxymethoxy)-5-methylbenzaldehyde (8)**

10-(3-bromo-2-(methoxymethoxy)-5-methylphenyl)anthracene(7) (1.9 g, 46.6 mmol) was taken in dry THF (50 ml) cooled to -78°C under nitrogen atmosphere, n-butyl lithium (2.5 M in hexane) (2.8 mL, 6.9 mmol) was added dropwise over a period of 30 min and allowed to warm to -50°C and stirred for 4 hr. Again cooled the reaction mass to -78°C, DMF (0.7 mL, 9.32 mmol) was added drop wise, The reaction was monitored by TLC, after completion, the reaction was quenched by the addition of water (10 mL) and then the reaction mixture was extracted with ethyl acetate (2×10 mL), washed with water (20 mL), saturated brine (10 mL) and dried the organic layer over anhydrous Na\(_2\)SO\(_4\) and concentrated under reduced pressure. Resulting crude was purified by flash column chromatography (silica gel (230-400 mesh), 15 % ethyl acetate in n-hexane as eluent) to afford 3-(anthracen-10-yl)-2-(methoxymethoxy)-5-methylbenzaldehyde (8) (1.2 g, 75 %) as yellow solid. \( ^1H \) NMR (CDCl\(_3\)/400 MHz); \( \delta \) 10.5 (1H, s, CHO), 8.55 (1H, s, H\(_{13}\)), 8.08-8.06 (2H, m, \( J = 7.92 \) Hz, H\(_{19}, H_{20}\)), 7.90 -7.89 (1H, d, H\(_4\)), 7.68-7.66 (2H, m, H\(_{17}, H_{18}\)), 7.52-7.48 (2H, m,
Synthesis of 2-(3-(anthracen-10-yl)-2-(methoxymethoxy)-5-methylbenzylamino) acetic acid (9a)

3-(Anthracen-10-yl)-2-(methoxymethoxy)-5-methylbenzaldehyde (8) (1g, 2.8 mmol) and glycine ethyl ester hydrochloride (1.1 eqv) were taken in ethanol (30 ml) stirred for 30 min and NaBH$_4$ (74 mg, 1.9 mmol) was added portionwise and stirred at room temperature for 8 h. The reaction was monitored by TLC, after completion of the reaction, it was quenched by the addition of 1.5 N HCl solution (10 mL) stirred for 30 min and extracted with ethyl acetate (2×10 mL), washed with water (20 mL), saturated brine (10 mL) and dried the organic layer over anhydrous MgSO$_4$ and concentrated to afford gummy liquid. The residue was purified by column chromatography on silica gel (60-120 mesh) with 14-18 % ethyl acetate in petroleumether as an eluent to get ethyl 2-(3-(anthracen-10-yl)-2-(methoxymethoxy)-5-methylbenzylamino) acetate (9a as ester) as yellowish oil (1.53 g, 78.4 %). LCMS m/z: 444.2 (M$^+$+1).

Lithium hydroxide (0.26 g, 10.9 mmol) was added to a mixture of ethyl 2-(3-(anthracen-10-yl)-2-(methoxymethoxy)-5-methylbenzylamino) acetate (9a as ester) (1 g, 1.8 mmol) in THF: methanol: water (1:1:1) and stirred at room temperature for 15 h. Completion of the reaction was confirmed by TLC. Reaction mass was concentrated as such and the residue was taken in small amount of water (5 ml), washed with diethyl ether (2×5 mL), remaining aqueous layer was acidified with 1.5 N HCl under cooled condition, product was extracted with DCM and triturated with ether to afford the (9a) 2-(3-(anthracen-10-yl)-2-(methoxymethoxy)-5-methylbenzylamino) acetic acid (0.8 g, 84.75 %) as off-white solid. mp: 130.6-131.8 °C. $^1$H NMR (CD$_3$OD /400 MHz); δ 8.61 (1H, s, H$_{13}$), 8.11-8.09 (2H, m, J=8.44 Hz, H$_{17}$, H$_{22}$), 7.61-7.59 (2H, d, J=8.72 Hz, H$_{19}$, H$_{23}$) , 7.53-7.47 (3H, m, H$_{2}$, H$_{18}$, H$_{21}$), 7.44-7.40 (2H, m, H$_{17}$, H$_{22}$), 7.24 (1H, d, J$_{18}$), 4.40 (2H, s, H$_{8}$), 4.17 (2H, s, H$_{24}$), 3.63 (2H, s, H$_{25}$), 2.85 (3H, s, H$_{9}$), 2.46 (3H, s, H$_{7}$) ppm. $^{13}$C NMR (CD$_3$OD /75 MHz); δ 173.2 (carbonyl carbon in COOEt), 154.2 (C6)
132.9, 132.4, 131.4, 129.7, 128.6, 127.2, 126.9, 126.4, 99.6 (C8), 56.8 (C9), 20.7 (C7) ppm. IR (KBr): 3438 (O-H), 2961 (N-H), 2823, 2457, 1736 (C=O), 1652, 1624.734 cm⁻¹. LCMS m/z: 416.2 (M⁺+1).

Mass spectrum of 9a ester
Synthesis of hydroxyl fluorescent amino acids (10a)
4.5 M HCl in dioxane (5 vol) was added dropwise to a solution of 2-(3-(anthracen-10-yl)-2-(methoxymethoxy)-5-methylbenzylamino) acetic acid (9a) in 1, 4-dioxane (10 vol), and the reaction mixture was stirred at room temperature for 5 h. Reaction progress was monitored by TLC. After completion of the reaction, the mixture was diluted with water (7 ml), the compound was extracted with ethyl acetate (2×10 mL) and the combined organic layer was washed with water (5 ml), brine (5 ml) and dried over Na₂SO₄ and concentrated under reduced pressure. The resulting crude was purified by column chromatography to get 2-(3-(anthracen-10-yl)-2-hydroxy-5-methylbenzylamino) acetic acid 10a as white solid (0.4 g, 87 %). Mp : 206.3-207.5°C. ¹H NMR (DMSO-d₆/300 MHz); δ 9.13 (2H, brs, OH in PhOH), 8.66 (1H, s, H₁₃), 8.14-8.12 (2H, m, J=8.37 Hz, H₁₉, H₂₀), 7.54-7.47 (4H, m, H₁₁, H₁₆, H₁₇, H₂₁), 7.42-7.37 (3H, m, H₁₂, H₁₈, H₂₂), 6.98 (1H, d, H₄), 4.28 (2H, s, H₂₄), 3.90 (2H, s, H₂₅), 2.29 (3H, s, H₇). ¹³C NMR (DMSO-d₆/100 MHz); δ 168.0 (Carbonyl C in COOH), 151.7 (C₆), 133.8 (C₁₃), 132.5 (C₁₁), 132.1 (C₃), 131.1 (C₄), 130.2, 128.4, 128.3, 126.7, 126.3, 125.7, 125.6, 119.3 (C₅), 46.5 (N-CH₂-COOH), 45.6 (Ar-CH₂-NH), 20.0 (C₇).IR (KBr): 3553 (O-H in PhOH), 3345 (O-H from COOH), 2917, 2748, 1714 (C=O), 1419, 1314 cm⁻¹. LCMS m/z: 372.2 (M⁺+1), HPLC purity; (94.60%).
Synthesis of fluorescent peptide acids (11a)

Compound 9b (0.8 g, 1.5 mmol) glycine ethyl ester hydrochloride (0.23 g, 1.7 mmol) and triethyl amine (0.32 mL, 2.3 mmol) was taken in dimethyl foramide (15 mL), stirred for 30 min followed by TBTU (0.49 g, 1.5 mmol) was added one lot and allowed to stirred at room temperature for 18 h. Reaction mass was concentrated as such, diluted with water and extracted with ethyl acetate (2x30 mL). The combined ethyl acetate extracts were washed with water (2x20 mL), brine solution (30 mL) and dried over Na₂SO₄. Resulting crude was purified by column chromatography to afford compound (11a as ester) (0.8 g, 86 %) as off-white solid. mp: 189.7-189.9 °C. H NMR (400 MHz, DMSO-ᴅ₆): δ 8.71 (1H, brs, CONH), 8.33-8.29 (3H, m, H₁₃, H₁₉, H₂₀), 7.53-7.47 (4H, m, H₁₈, H₂₁, H₁₆, H₂₃), 7.44-7.4 (2H, m, H₁₇, H₂₂), 7.35 (1H, s, H₁), 7.15-7.05 (2H, d, J=8.2 Hz, H₂₈, H₃₂), 7.24 (1H, s, H₄), 6.85-6.81 (2H, d, J=8.2 Hz, H₂₉, H₃₁), 4.25-4.19 (2H, q, J=7.48 Hz, CH₂CH₃), 4.08 (2H, s, H₇), 3.89-3.74 (4H, m, H₂₄, H₃₄), 3.52 (1H, s, H₂₅), 3.14-3.11 (1H, m, H₂₆), 3.00-2.98 (1H, m, H₂₆), 2.06-2.05 (6H, s, H₇, H₉), 1.51 (3H, t, J=7.48 Hz, CH₃CH₃). LCMS: m/z 607.3.
A solution of intermediate 11a ester (0.7 g, 1.2 mmol) in THF: Methanol: water (1:1:1) was added lithium hydroxide (0.16 g, 6.9 mmol), stirred at room temperature for 16 h. Reaction mass was concentrated and the remaining aqueous mixture was washed with diethyl ether (2x20 mL) and acidified with 1.5 N hydrochloric acid solution under cooled condition. Then the reaction mass was extracted with dichloromethane (2x30 mL) and concentrate to half of its volume and titrated with ether (50 mL) to afford 11a (0.6 g, 90.2 %) as off-white solid. mp: 187.3-188.9 °C.

\[ \text{11a} (\text{Ester}) \]

\(^1\text{H} \text{NMR} (400 \text{ MHz, DMSO}-d_6): \delta 9.32 (1H, brs, Phenolic OH), 8.63 (1H, brs, CONH), 8.11-8.09 (3H, m, H_{13}, H_{19}, H_{20}), 7.53-7.40 (6H, m, H_{16}, H_{17}, H_{18}, H_{21}, H_{22}, H_{23}), 7.2 (1H, s, H_2), 7.05-7.03 (2H, d, J=8.2 Hz, H_{28}, H_{32}) \]
6.92 (1H, s, H4), 6.66–6.64 (2H, d, J=8.2 Hz, H29, H31), 4.06 (2H, s, H3), 3.81–3.65 (4H, m, H24, H43), 3.33–3.29 (1H, m, H25), 2.89–2.86 (1H, m, H26), 2.61 (1H, m, H28), 2.27 (3H, s, H9), 2.26 (3H, s, H7). 13C (100 MHz, DMSO-d6): δ 173.4 (C35), 171.4 (C33), 155.9 (C30), 151.3 (C6), 133.7 (C13), 133.3 (C10), 133.2 (C27), 131.6 (C3), 130.9 (C12, C14), 130.8 (C2), 130.2 (C4), 130.1 (C11, C15), 129.7 (C28, C32), 128.5 (C1), 128.3 (C20, C23), 126.6 (C5), 126.4 (C16, C19), 125.8 (C17, C18), 125.3 (C21, C22), 115.9 (C29, C31), 98 (C8), 63.4 (C25), 55.4 (C9), 46.1, 38.8 (C26), 20.4 (C7). IR (KBr): 3759 (O-H), 3276 (N-H), 3051, 2924, 2853, 2065, 1700 (C=O), 1593 (C=ONH), 1514, 1397, 1397, 1226, 1154, 1069, 958, 883, 736 cm⁻¹. LCMS: m/z 579.3 (M⁺+1).
13C NMR spectrum of 11a

IR spectrum of 11a
LCMS Report

Data file: 
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Injection Date: 
Injection Vol.: 2µL
Sample Name: 
Acq Method: C:\CHEM32\V\METHODS\ZX_109MFA-VE.M

Method info: Mobile Phase: A: 0.1%HOAc in water, B: Methanol Flow: 1.2ml/min
Column: Zorbax C18 (50x4.6mm-5µ) SC/LC/309, POSITIVE MODE
Time (min): 0-----3.5 3.5------5.0 5.0------5.5 5.5------7.0
%B: 10-95 95 95-10 10

*Maximum Chromatogram of D

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Mass spectrum of 11a
Fluorescence spectra

The selected anthracene amino acids were evaluated for their UV absorption and fluorescence emission spectrum (Figure-3) similar to the procedures available in literature 21.

In UV absorption spectrum the characteristic anthracene absorption and emission were observed with corresponding excitation wavelength. Fluorescence spectra of some selected compounds were depicted in figure-2.

Fluorescent labeling of cells

The synthesized fluorescent amino acids were taken under study for biomarking the cancer cells. The fluorescent amino acids 9b were found to be helpful for fluorescently marking breast cancer MCF7 cells with blue fluorescence (Figure 3). The fluorescent labeling of the MCF7 cells after incubation with fluorescent amino acids can be due to the interaction of fluorescent amino acid group interacting with amine groups of cell wall and intracellular proteins.19a The chosen compounds also exhibited mild antibacterial activity against Gram +ve and Gram –ve bacteria.
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Cell line and culture
Human Breast cancer MCF-7 cell lines were obtained from King Institute of Preventive Medicine and Research, Guindy, Chennai. The cells were maintained in Minimal Essential Media supplemented with 10% FBS, penicillin (100 U/mL), and streptomycin (100 μg/mL) in a humidified atmosphere of 50 μg/mL CO₂ at 37 °C.

Antimicrobial activity of selected fluorescent amino acids/peptide
At 10mg/ml concentration (~25mM concentration), growth inhibition measured in terms of zone of inhibition demonstrated that growth is inhibited for both Gram +ve and Gram –ve organisms and one yeast cells with reference to selected fluorescent amino acids. Maximum inhibition was observed with Gram +ve compared to Gram –ve. Among the Gram –ve’s E.coli and Shigella were inhibited at higher level compared to Pseudomonas and Proteus species.

Table-I Antimicrobial activity of some selected fluorescent amino acids

<table>
<thead>
<tr>
<th>Compound</th>
<th>Zone of inhibition (mm) at 10 mg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Micrococcus luteus</td>
</tr>
<tr>
<td>9b</td>
<td>11.5 ± 0.16</td>
</tr>
<tr>
<td>10b</td>
<td>13 ± 0.21</td>
</tr>
<tr>
<td>10c</td>
<td>20 ± 0.47</td>
</tr>
<tr>
<td>10d</td>
<td>12 ± 0.42</td>
</tr>
<tr>
<td>11a</td>
<td>12.5 ± 0.11</td>
</tr>
<tr>
<td>11b</td>
<td>11.5 ± 0.13</td>
</tr>
</tbody>
</table>

Among the Gram +ve no significant difference was observed between the Bacillus genera and growth inhibition was less with Staphylococcus aureus. Among the selected organisms, maximum growth inhibition was observed with Micrococcus and Candida albicans. Table-I represents the results on zone of inhibition measured in mm along with sample ID.

Conclusion
The synthesized anthracene based fluorescent amino acids were studied with fluorescence imaging of MCF7 cell lines. MCF7 cells were stained with blue fluorescence and the anthracene amino acids hold promise as fluorescent imaging tools for biological samples. Though the small molecule amino acid fluorescent label helps in labeling the cells, we still need to ascertain the mechanism with further binding studies with cell surface proteins and intracellular proteins which is future research interest. Moreover the fluorescent amino acids show antimicrobial growth inhibition apart from fluorescent imaging. This unique fluorescent property of the amino acid will enable us study the mechanism involved in microbial inhibition of fluorescent peptides in near future.

REFERENCES
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