RESULTS AND DISCUSSION

CHAPTER-I

Synthesis and characterization of fluorescent anthracenyl sensors

Symyx technologies 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 developed a new synthetic procedure for the synthesis of MOM protected anthracenyl aldehyde with simple purification techniques.

The protection of phenolic group in 2-bromo-4-methyl phenol (37) was carried with methoxy methyl chloride, diisopropyl ethyl amine and DCM at room temperature over a period of 4 h to afford intermediate 38 with good yield. Without any purification, 38 was taken as such to the next step for suzuki coupling. Resulting reaction crude was treated with anthracene boronic acid (39), sodium carbonate, and tetrakis(triphenylphosphine)palladium in toluene at 110 ºC for 8 h to afford 40. Same suzuki reaction was also attempted by taking reverse analogues like 9-bromo anthracene and 2-(methoxymethoxy)-5-methylphenylboronic acid with the same condition but we observed less yield, MOM cleavage was carried out by treating 40 with 4.5 M HCl in dioxane at 0 ºC to room temperature for 5 h to afford 2-anthracenyl phenolic compound (41).
Scheme-2

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

Bromination of 41 was carried out by the treatment with NBS, triethyl amine and DCM as solvent at room temperature for 8 h to afford (42) anthracenyl bromo phenol. The same reaction was also attempted by using Br₂/CHCl₃ and Br₂/AcOH at 0 °C to room temperature, but the reaction resulted in numerous products as monitored by TLC. Compound 42 was protected with MOMCl using Hunig’s base and DCM as solvent for 4 h to get analytically pure MOM protected bromo compound 43 after column purification. Formylation of 43 was attempted by using n-BuLi and DMF at -78°C to afford 3-(anthracen-10-yl)-2-(methoxymethoxy)-5-methylbenzaldehyde (44) as yellow solid.

2-Bromo-1-(methoxymethoxy)-4-methylbenzene (38)
Yield : 96 %

Nature : Yellowish oil

**¹H NMR** (CDCl₃/300 MHz); δ 7.36 (1H, s, H₃), 7.08 -7.05 (2H, m, H₆, H₅), 4.32 (2H, s, H₈), 3.24 (3H, s, H₉), 2.30 (s, 3H, H₇) ppm.

**¹³C NMR** (CDCl₃/75 MHz); δ 151.3 (C₁), 134.5 (C₃), 132.9 (C₄), 129.1 (C₅), 116.4 (C₆), 112.1 (C₂), 94.3 (C₈), 55.3 (C₉), 23.1 (C₇) ppm.

**LCMS purity**; purity (96.20 %), method- ZX_1090MFA+VE.M, RT- 4.635 min.

**Anthracen-10-ylboronic acid (39)**

![Anthracen-10-ylboronic acid (39)](image)

Yield : 89 %

Nature : Yellow solid

**¹H NMR** (CDCl₃/300 MHz); δ 8.47 (s, 1H, H₈), 8.14-8.1 (m, 4H, H₃, H₆), 7.53-7.46 (m, 4H, H₄, H₅), 5.11 (s, 2H, OH) ppm.

**10-(2-(Methoxymethoxy)-5-methylphenyl)anthracene (40)**

![10-(2-(Methoxymethoxy)-5-methylphenyl)anthracene (40)](image)

Yield : 92 %

Nature : White solid
$^1$H NMR (CDCl$_3$/300 MHz); 8.49 (s, 1H, H$_1$), 8.06-8.03 (d, 2H, $J$=8.43 Hz, H$_{2a}$), 7.68-7.68 (d, 2H, $J$=8.42 Hz, H$_{2d}$), 7.48-7.43 (m, 2H, H$_{2b}$), 7.38-7.1 (m, 4H, H$_{2c}$, H$_8$, H$_9$), 7.10 (m, 1H, H$_6$), 4.90 (s, 2H, H$_{12}$), 3.22 (s, 3H, H$_{13}$), 2.34 (s, 3H, H$_{11}$) ppm.

**LCMS purity:** purity (97.91 %), Method- ZX_1090MFA.M, RT-5.306 min.

**2-(Anthracen-10-yl)-4-methylphenol (41)**

![Structure of 2-(Anthracen-10-yl)-4-methylphenol (41)]

**Yield:** 95 %

**Nature:** White solid

$^1$H NMR (CDCl$_3$/ 400 MHz); 8.57 (1H, s, H$_1$), 8.09-8.07 (2H, m, H$_{2a}$), 7.72-7.69 (2H, m, H$_{2d}$), 7.52-7.48 (2H, m, H$_{2b}$), 7.44-7.40 (2H, m, H$_{2c}$), 7.29 (1H, m, H$_6$), 7.08-7.06 (2H, m, H$_8$, H$_9$), 4.37 (1H, s, OH, H$_{12}$), 2.38 (3H, s, H$_{11}$) ppm.

**LCMS exhibited the molecular ion peak at EIMS m/z:** 285.4 (M$^+$+1).

**UPLC purity:** purity (96.10 %), method- ZX_1090MFA.M, RT- 1.43 min.

**2-(Anthracen-10-yl)-6-bromo-4-methylphenol (42)**

![Structure of 2-(Anthracen-10-yl)-6-bromo-4-methylphenol (42)]

**Yield:** 82 %
Nature: Off-white solid

\textsuperscript{1}H NMR (CDCl\textsubscript{3}/ 300 MHz); \(\delta\) 8.56 (1H, s, H\textsubscript{1}), 8.09-8.06 (2H, d, \(J=8.37\) Hz, H\textsubscript{2a}), 7.66-7.53 (2H, d, \(J=8.76\) Hz, H\textsubscript{2d}), 7.53-7.27 (5H, m, H\textsubscript{2b}, H\textsubscript{2c}, H\textsubscript{6}), 7.05 (1H, s, H\textsubscript{8}), 5.08 (1H, s, OH, H\textsubscript{12}), 2.37 (3H, s, H\textsubscript{11}) ppm.

LCMS exhibited the molecular ion peak at EIMS \textit{m/z}: 361.1 (M\textsuperscript{+}-2).

10-(3-Bromo-2-(methoxymethoxy)-5-methylphenyl)anthracene (43)

Yield: 97%

Nature: Off-white solid

\textsuperscript{1}H NMR (CDCl\textsubscript{3}/ 300 MHz); \(\delta\) 8.51 (1H, s, H\textsubscript{1}), 8.05-8.02 (2H, d, \(J=8.13\) Hz, H\textsubscript{2a}), 7.68-7.60 (3H, m, H\textsubscript{2d}, H\textsubscript{6})), 7.50-7.38 (4H, m, H\textsubscript{2b}, H\textsubscript{2c}), 7.11-7.1 (1H, d, H\textsubscript{8}), 4.47 (2H, s, H\textsubscript{12}), 2.39 (3H, s, H\textsubscript{13}), 2.30 (3H, s, H\textsubscript{11}) ppm.

LCMS exhibited the molecular ion peak at EIMS \textit{m/z}: 409.1 (M\textsuperscript{+}+2).

UPLC purity; purity (99.61 %), method- ZX\_1090MFA.M, RT- 5.25 min.

3-(Anthracen-10-yl)-2-(methoxymethoxy)-5-methylbenzaldehyde (44)
Yield: 76%

Nature: Yellowish solid

$^1$H NMR spectral reports of 44: (CDCl$_3$/400 MHz); δ 10.5 (1H, s, H$_{14}$), 8.55 (1H, s, H$_1$), 8.08 - 8.06 (2H, m, J = 7.92 Hz, H$_{2a}$), 7.90 - 7.89 (1H, d, H$_8$), 7.68 - 7.66 (2H, m, H$_{2d}$), 7.52 - 7.48 (2H, m, H$_{2b}$), 7.45 - 7.42 (3H, m, H$_{2c}$, H$_6$), 4.38 (2H, s, H$_{12}$), 3.24 (3H, s, H$_{13}$), 2.35 (3H, s, H$_{11}$) ppm.

LCMS exhibited the molecular ion peak at EIMS $m/z$: 355.3 (M$^+$-1).

Scheme-3

Synthesis of anthracenyl fluorescent amino acids

Compound 44 was taken for reductive amination with different amino acids (45a-d) using sodium borohydride in ethanol under nitrogen atmosphere for 8 h to afford crude fluorescent amino esters, which were purified by column chromatography to get analytically pure oily product 46(a-d). Hydrolysis of compound 46(a-d) was carried out by using 4 equiv of lithium hydroxide and mixture of solvents
(tetrahydrofuran: methanol: water) at room temperature for 15 h to get 47(a-d). All the synthesized compounds were characterized by $^1$H NMR, $^{13}$C NMR, LCMS and HPLC. Ethy2-(3-(anthracen-10-yl)-2-(methoxymethoxy)-5-methylbenzylamino) acetate (46a)

![Structure of 46a](image)

**Yield**: 74 %

**Nature**: Yellowish viscous oil

$^1$H NMR (CDCl$_3$/300 MHz); δ 8.51(1H, s, H$_1$), 8.06-8.03 (2H, m, $J$=8.31 Hz, H$_{2a}$), 7.68-7.65 (2H, d, $J$=8.52 Hz, H$_{2d}$), 7.48-7.40 (5H, m, H$_{2b}$, H$_{2c}$, H$_6$), 7.27 (1H, d, H$_8$), 4.25 (2H, s, H$_{12}$), 4.22 (2H, q, $J$=7.12 Hz, H$_{18}$), 4.12 (2H, s, H$_{14}$), 3.67 (2H, s, H$_{16}$),2.75 (3H, s, H$_{13}$), 2.40 (3H, s, H$_{11}$), 1.32 (3H, t, $J$=7.12 Hz, H$_{19}$) ppm.

$^{13}$C NMR (CDCl$_3$/75 MHz); δ 169.6 (C17), 151.2 (C10), 138.6, 134.3, 133.8, 133.6, 133.1, 132.7, 132.7, 131.6, 129.4, 128.5, 127.1, 127.1, 126.9, 122.6, 99.6 (C12), 61.1 (C18), 55.8 (C13), 49.2 (C16) 44.8 (C14), 23.7 (C11), 14.2 (C19) ppm.

**LCMS** exhibited the molecular ion peak at EIMS m/z: 444.2 (M$^+$$+1$).

**UPLC purity**: purity (92.06 %), method- ZX_1090MFA.M, RT- 3.963 min.
Ethyl 2-(3-(anthracen-10-yl)-2-(methoxymethoxy)-5-methylbenzylamino) propanoate (46b)

Yield : 90 %

Nature : White solid

$^1$H NMR (CDCl$_3$/400 MHz); $\delta$ 8.50 (1H, s, $H_1$), 8.05-8.03 (2H, m, $H_{2a}$), 7.76-7.69 (2H, m, $H_{2d}$), 7.49-7.39 (5H, $H_{2b}$, $H_{2c}$), 7.06 (1H, d, $H_8$), 4.29-4.27 (2H, m, $H_{12}$), 3.99-3.90 (2H, dd, $J=13$ Hz, $H_{14}$), 3.97 (2H, t, $J=7.12$ Hz, $H_{19}$), 3.44 (1H, q, $J=6.9$ Hz, $H_{16}$), 2.56 (3H, s, $H_{13}$), 2.41 (3H, s, $H_{11}$), 1.27 (3H, d, $H_{17}$), 1.28 (3H, t, $H_{20}$).

$^{13}$C NMR (CDCl$_3$/100 MHz); $\delta$ 170.5 (C18), 151.1 (C10), 137.9, 134.5, 132.2, 131.1, 131.3, 130.9, 130.7, 130.9, 129.6, 128.2, 127.6, 126.1, 125.9, 125.4, 122.7, 97.7 (C12), 58.5 (C19), 56.7 (C16), 55.7 (C13), 40.3 (C14), 24.9 (C11), 17.7 (C17), 14.3 (C20) ppm.

LCMS exhibited the molecular ion peak at EIMS $m/z$: 458.6 (M$^+$+1).

UPLC purity; purity (87.64 %), method- ZX_1090MFA.M, RT- 4.724 min.

(S)-Ethyl 2-(3-(anthracen-10-yl)-2-(methoxymethoxy)-5-methylbenzylamino)-3-(4-hydroxyphenyl)propanoate (46c)
Yield : 85 %

Nature : Brownish viscous oil

\( \text{mp} : 186.5-186.7 \, ^\circ \text{C} \)

\(^1\text{H} \text{NMR} \) (DMSO-\(d_6/300 \text{ MHz} \)); \( \delta \) 9.19 (1H, s, H\(_{22}\)), 8.64 (1H, m, H\(_1\)), 8.12-8.09 (2H, m, H\(_{2a}\)), 7.52-7.42 (6H, m, H\(_{2b}, \, \text{H}_c, \, \text{H}_{2e}\)), 7.27 (1H, m, H\(_6\)), 7.08-7.06 (2H, m, H\(_{19}\)), 7.01 (1H, s, H\(_8\)), 6.67-6.44 (2H, d, H\(_{20}\)), 4.12 (2H, s, H\(_{12}\)), 4.22 (2H, q, \( J = 7.12 \text{ Hz}, \) H\(_{24}\)), 3.54-3.51 (2H, m, H\(_{14}\)), 3.23-3.29 (1H, s, H\(_{16}\)), 2.79 (2H, d, H\(_{17}\)), 2.31 (3H, s, H\(_{13}\)), 2.29 (3H, s, H\(_{11}\)), 1.33 (3H, t, \( J = 7.12 \text{ Hz}, \) H\(_{25}\)) ppm.

\(^{13}\text{C} \text{NMR} \) (DMSO-\(d_6/100 \text{ MHz} \)); \( \delta \) 171.6 (C\(_{23}\)), 155.2 (C\(_{21}\)), 151.5 (C\(_{10}\)), 134.3, 133.9, 133.7, 131.5, 131.3, 130.4, 130.3, 129.5, 128.2, 126.9, 126.8, 126.3, 126.3, 126.1 (C\(_5\)), 125.5, 115.3, 98.4 (C\(_{12}\)), 62.4 (C\(_{16}\)), 55.8 (C\(_{24}\)), 48.2 (C\(_{13}\)), 45.9 (C\(_{14}\)), 36.7 (C\(_{17}\)), 26.6 (C\(_{11}\)), 14.4 (C\(_{25}\)) ppm.

LCMS exhibited the molecular ion peak at EIMS \( m/z \): 547.4 (M\(^+\)+1).

**UPLC purity**: purity (97.14 %), method- ZX_1090MFA.M, RT- 4.006 min.

**Ethyl 2-(3-(anthracen-10-yl)-2-(methoxymethoxy)-5-methyl benzyl amino)-3-hydroxy propanoate (46d)**

Yield : 85 %

Nature : White solid
mp : 212.4-212.6 °C

$^1$H NMR (CDCl$_3$/400 MHz); δ 8.52 (1H, s, H$_1$), 8.05-8.03 (2H, m, H$_{2a}$), 7.71-7.67 (2H, m, H$_{2d}$), 7.48-7.39 (4H, m, H$_{2b}$, H$_{2c}$), 7.33-7.32 (1H, d, H$_6$), 7.07 (1H, d, H$_8$), 4.24-4.18 (2H, dd, J = 9.48 Hz, H$_{12}$), 3.97 (2H, q, J=7.12 Hz, H$_{20}$), 3.88-3.86 (1H, dd, H$_{14}$), 3.76 (1H, m, H$_{14}$), 3.72-3.59 (2H, m, H$_{17}$), 3.59-3.57 (1H, t, J = 4.8 Hz, H$_{16}$), 2.66 (3H, s, H$_{11}$), 1.28 (3H, t, J=7.12 Hz, H$_{21}$) ppm.

$^{13}$C NMR (CDCl$_3$/100 MHz); δ 170.1 (C$_{19}$), 150.8 (C$_{10}$), 134.5, 132.9, 132.5, 131.9, 131.6, 130.3, 130.3, 130, 128.9, 128.6, 127.9, 126.3, 126.1, 126.0, 125.7(C5), 123.3, 96.6 (C$_{12}$), 62.9 (C$_{16}$), 61.1 (C$_{17}$), 61.3 (C$_{20}$), 56.7 (C$_{13}$), 44.7 (C$_{14}$), 29.6 (C$_{11}$), 14.1 (C$_{21}$) ppm.

LCMS exhibited the molecular ion peak at EIMS m/z: 474.2 (M$^+$+1).

UPLC purity; purity (97.41 %), method- ZX_1090MFA.M, RT- 3.879 min.

2-(3-(Anthracen-10-yl)-2-(methoxymethoxy)-5-methyl benzyl amino)-3-(4-hydroxy phenyl) propanoic acid (47c)

Yield : 90 %

Nature : White solid

mp : 183.8-185.2 °C

$^1$H NMR spectral reports of 47c: (DMSO-d$_6$/400 MHz); δ 9.4 (1H, brs, H$_{22}$), 8.66 (1H, m, H$_1$), 8.13 (2H, m, H$_{2a}$), 7.55-7.40 (6H, m, H$_{2b}$, H$_{2c}$, H$_{2d}$), 7.36 (1H, m, H$_6$), 7.08-7.06 (2H, m, H$_{19}$), 7.02 (1H, s, H$_8$), 6.69-6.67 (2H, d, J=8.4 Hz, H$_{20}$), 4.13-4.11 (2H, dd, H$_{12}$), 4.00-3.97
(1H, d, \(J_{\text{Hc,Hd}} = 13.8\) Hz, \(\text{H}_{14}\)), 3.90-3.86 (1H, d, \(J_{\text{Hd,Hc}} = 13.8\) Hz, \(\text{H}_{14}\)), 3.57-3.54 (1H, m, \(\text{H}_{16}\)), 2.95-2.89 (2H, m, \(\text{H}_{17}\)), 2.50 (3H, s, \(\text{H}_{13}\)), 2.33 (3H, s, \(\text{H}_{11}\)) ppm.

\(^{13}\text{C}\) NMR spectral reports of 47c: (DMSO-\text{d}_6/100 MHz); \(\delta\) 173.4 (C23), 156.1 (C21), 151.5, 133.4, 132.9, 132.5, 131.1, 130.9, 130.7, 130.3, 129.7, 128.4, 127.6, 126.8, 126.3, 126.3, 125.9, 125.3, 115.1, 98.2 (C12), 62.3 (C16), 55.6 (C13), 45.6 (C14), 36.9 (C17), 26.8 (C11) ppm.

IR (KBr): 3410 (OH, \(\text{H}_{22}\)), 3175 (NH, \(\text{H}_{15}\)), 2974 (C-H), 2820 (OH, \(\text{H}_{24}\)), 1613 (C=O), 1225 cm\(^{-1}\).

LCMS exhibited the molecular ion peak at EIMS \(m/z\): 522.3 (M\(^+\)+1).

UPLC purity; purity (99.85 %), method- ZX_1090MFA.M, RT- 4.256 min.

\(^1\text{H}\) NMR spectral analysis of 47c: \(^1\text{H}\) NMR spectrum showed a singlet at \(\delta\) 2.33 which corresponds to methyl group (\(\text{H}_{11}\)) attached to the aromatic ring, peak at \(\delta\) 2.50 corresponds to methyl group attached with MOM protecting group (-OCH\(_2\)OCH\(_3\), \(\text{H}_13\)), doublet of doublet at \(\delta\) 2.95-2.89 is due to (\(\text{H}_{17}\)) (-CH\(_2\)-) group attached to tyrosinyl amino acid, multiplet at \(\delta\) 3.57-3.54 corresponds to methine group (\(\text{H}_{16}\)) in tyrosine amino acid moiety (N-CH-COOH), a doublet of doublet peak at \(\delta\) 4.00-3.97 and 3.90-3.86 due to geminal protons (\(\text{H}_{14}\)) attached to C14 whose coupling constant values are \(J_{\text{Hc,Hd}} = 13.8\) Hz, \(J_{\text{Hd,Hc}} = 13.8\) Hz) respectively, a doublet of doublet peak at 4.13-4.11 due to geminal protons (\(\text{H}_{12}\)) whose coupling constant values are \(J_{\text{Ha,Hb}} = 5.96\) Hz, \(J_{\text{Hb,Ha}} = 5.96\) Hz) respectively, and a peak for \(\text{H}_{20}\) resonated as doublet at \(\delta\) 6.69-6.67 with \(J_{\text{H19,H20}} = 8.4\) Hz, at \(\delta\) 7.02 a single peak resonated due to \(\text{H}_{8}\), a peak for \(\text{H}_{19}\) was observed at \(\delta\) 7.08-7.06 as multiplet due to ortho coupling with \(\text{H}_{20}\) proton, \(\delta\) 7.36 corresponds to (\(\text{H}_6\)) proton attached to the aromatic ring, \(\text{H}_{2b}, \text{H}_{2c}, \text{H}_{2d}\) anthracenyl protons were resonates at \(\delta\) 7.55-7.40 ppm as multiplet which was due to ortho and meta coupling, a peak at \(\delta\) 8.13 was observed as multiplet corresponds to anthracenyl...
protons H\textsubscript{2a}, δ 8.66 a multiplet peak was corresponds to H\textsubscript{1}, a broad singlet peak at 9.4 due to phenolic OH proton (H\textsubscript{22}).

\textbf{\textsuperscript{13}C NMR spectral reports of 47c:} The spectrum displayed 25 signals; a signal at δ 26.8 (Ar-CH\textsubscript{3}) is due to methyl carbon attached to the phenyl ring, a signal at δ 36.9 corresponding to methylene carbon of tyrosine moiety CH\textsubscript{2}-PHOH, a signal at δ 45.6 corresponding to benzyl (Ph-CH\textsubscript{2}-NH) carbon C\textsubscript{14}, a signal at δ 55.6 (-OCH\textsubscript{2}OCH\textsubscript{3}) is due to methyl carbon in MOM group, a signal at 62.3 corresponds to methine carbon (N-CH-COOH) in tyrosine moiety, a signal at δ 98.2 (-OCH\textsubscript{2}OCH\textsubscript{3}) is due to methylene carbon in MOM protecting group, similarly signals at 132.9 (C\textsubscript{4}), 132.5 (C\textsubscript{18}), 131.1 (C\textsubscript{7}), 130.9 (C\textsubscript{6}), 130.7, 130.3, 129.7, 128.4, 127.6, 126.8, 126.3, 125.9 (C\textsubscript{5}), 125.3 (C\textsubscript{9}), 115.1 (C\textsubscript{20}) were due to aromatic carbons, a signal at 151.5 corresponds to C\textsubscript{10} carbon, a signal at 133.4 is due to anthracene C\textsubscript{1} carbon, a signal at 156.1 (C\textsubscript{21}-OH) is due to OH group attached carbon, a signal at 173.4 corresponds to acid group carbon (COOH).

\textbf{IR spectral analysis of 47c:}

The IR spectrum of 47c showed a sharp strong band at υ = 3410 cm\textsuperscript{-1} (OH stretching frequency of phenolic group), a strong band at υ=3175 cm\textsuperscript{-1} (NH stretching frequency of secondary amine), a strong band at υ=2974 cm\textsuperscript{-1} (C-H stretching frequency of methyl group), a strong band at υ=2820 cm\textsuperscript{-1} (stretching frequency of OH group in carboxylic acid group), a strong band at υ=1613 cm\textsuperscript{-1} (C=O stretching frequency of carboxylic acid group), a sharp strong band at υ=1514 cm\textsuperscript{-1} (stretching frequency of aromatic C=C group), a strong band at υ = 1225 cm\textsuperscript{-1} (C-O stretching frequency of ether and phenolic group).

Compound 47c showed the molecular ion peak at 522.3 (M\textsuperscript{+}+1) in LCMS consistent with the molecular formula C\textsubscript{33}H\textsubscript{31}NO\textsubscript{5}. 
2-(3-(Anthracen-10-yl)-2-(methoxymethoxy)-5-methylbenzylamino)acetic acid (47a)

Yield : 83 %
Nature : Off-White solid
mp : 130.6 -131.8 °C

\(^1\)H NMR (CD\(_3\)OD /400 MHz); \(\delta\) 8.61(1H, s, H\(_1\)), 8.11-8.09 (2H, m, \(J=8.44\) Hz, H\(_{2a}\)), 7.61-7.59 (2H, d, \(J=8.72\) Hz, H\(_{2d}\)), 7.53-7.47 (3H, m, H\(_{2b}\), H\(_{6}\)), 7.44-7.40 (2H, m, H\(_{2c}\)), 7.24 (1H, d, H\(_8\)), 4.40 (2H, s, H\(_{12}\)), 4.17 (2H, s, H\(_{14}\)), 3.63 (2H, s, H\(_{16}\)), 2.85 (3H, s, H\(_{13}\)), 2.46 (3H, s, H\(_{11}\)) ppm.

\(^{13}\)C NMR (CD\(_3\)OD /75 MHz); \(\delta\) 173.2 (C\(_{17}\)), 154.2 (C\(_{10}\)), 136.8, 136.3, 133.4, 133.3, 133.1, 132.9, 132.4, 131.4, 129.7, 128.6, 127.2, 126.9, 126.4, 99.6 (C\(_{12}\)), 56.8 (C\(_{13}\)), 51.4 (C\(_{16}\)), 44.8 (C\(_{14}\)), 20.7 (C\(_{11}\)) ppm.

IR (KBr): 3438 (O-H), 2961 (N-H), 2823, 2457, 1736 (C=O), 1652, 1624,734 cm\(^{-1}\).

LCMS exhibited the molecular ion peak at EIMS \(m/z\): 416.2 (M\(^+\)+1).

UPLC purity; purity (99.77 %), method- ZX_1090MFA.M, RT- 4.264 min.

Analytica data for 2-(3-(Anthracen-10-yl)-2-(methoxymethoxy)-5-methylbenzylamino)3-(4-hydroxy phenyl) propanoic acid (47c) have been discussed above.
2-(3-(Anthracen-10-yl)-2-(methoxymethoxy)-5-methylbenzylamino) propanoic acid

(47b)

Yield : 89 %

Nature : White solid

mp : 181.7-183.4 °C

\(^1\)H NMR (DMSO-d\(_6\)/400 MHz); \(\delta\) 8.69 (1H, s, H\(_1\)), 8.16-8.13 (2H, d, \(J=12\) Hz, H\(_{2a}\)), 7.58-7.51 (5H, m, H\(_6\), H\(_{2d}\), H\(_{2b}\)), 7.46-7.42 (2H, m, H\(_{2c}\)), 7.09 (1H, s, H\(_8\)), 4.23-4.19 (2H, s, H\(_{12}\)), 4.12-3.99 (2H, dd, \(J=12\) Hz, \(J=24\) Hz, H\(_{14}\)), 3.36-3.35 (1H, q, \(J=4\) Hz, H\(_{16}\)), 2.45 (3H, s, H\(_{13}\)), 2.38 (3H, s, H\(_{11}\)), 1.36 (3H, d, \(J=8\) Hz, H\(_{17}\)).

\(^{13}\)C NMR (DMSO-d\(_6\)/100 MHz); \(\delta\) 173.0 (C\(_{18}\)), 152.1 (C\(_{10}\)), 133.9 (C\(_1\)), 133.3 (C\(_4\)), 133.2, 131.6, 131.3, 130.5, 130.1, 128.8, 127.3, 126.7, 126.6, 126.3, 125.7, 98.7 (C\(_{12}\)), 57.1 (C\(_{16}\)), 56.2 (C\(_{13}\)), 45.3 (C\(_{14}\)), 20.8 (C\(_{11}\)), 17.3 (C\(_{17}\)).

IR (KBr): 3391(O-H), 2931(N-H), 1603(C=O), 1449, 1351, 1293, 1153, 953 cm\(^{-1}\).

LCMS exhibited the molecular ion peak at EIMS \(m/z\): 430.2 (M\(^+\)+1).

UPLC purity; purity (94.60 %), method- ZX_1090MFAPVE.M, RT- 4.250 min.
2-(3-(Anthracen-10-yl)-2-(methoxymethoxy)-5-methylbenzylamino)-3-hydroxy propanoic acid (47d)

Yield : 87 %
Nature : White solid
mp : 199.9-201.2 °C

$^1$H NMR (DMSO-d$_6$/400 MHz); δ 8.69 (1H, s, H$_1$), 8.16-8.14 (2H, d, J=8.4 Hz, H$_{2a}$), 7.59-7.51 (5H, m, H$_{2d}$, H$_{2b}$, H$_6$), 7.46-7.43 (2H, m, H$_{2e}$), 7.09 (1H, d, H$_8$), 4.20 (2H, s, H$_{12}$), 4.16-4.05 (2H, dd, J=6.8 Hz, H$_{17}$), 3.82-3.38 (2H, m, J=6.8 Hz, H$_{14}$), 3.40 (1H, t, J=4.8 Hz, H$_{16}$), 2.51 (3H, s, H$_{13}$), 2.29 (3H, s, H$_{11}$) ppm.

$^{13}$C NMR (DMSO-d$_6$/100 MHz); δ 170.5 (C$_{19}$), 151.7 (C$_{10}$), 133.5 (C$_1$), 133 (C$_4$), 132.7, 131.2, 131.1, 130.8, 130.1, 129.6, 128.4, 126.8, 126.4, 126.2, 125.9 (C$_5$), 125.3 (C$_9$), 98.2 (C$_{12}$), 62.9 (C$_{16}$), 61.1 (C$_{17}$), 55.7 (C$_{13}$), 45.8 (C$_{14}$), 20.3 (C$_{11}$) ppm.

IR (KBr): 3205 (O-H), 3056 (N-H), 2941(C-H), 2361, 1970 (C=O), 1619, 1384, 739 cm$^{-1}$.

LCMS exhibited the molecular ion peak at EIMS $m/z$: 446.1 (M$^+$+1).

UPLC purity; purity (87.64 %), method- ZX_1090MFA.M, RT- 4.762 min.
Synthesis of anthracenyl peptide derivatives

Probes having more binding sites will bind the metal ions effectively. In order to develop the more ligating property of fluorescent sensor, anthracenyl fluorescent amino acid moiety was extended by peptide formation which could be useful for binding different metal ions.

Scheme-4

The anthracenyl peptide derivatives (49a-b) were synthesised by reacting anthracene-L-Tyrosinyl amino acid derivative 47c with different racemic amino esters (glycine and valine ethyl ester) using TBTU in DMF as a solvent at room temperature for 16 h to afford 48 (a-b), resulting esters were hydrolysed by using lithium hydroxide at room temperature with good yield. All the synthesized products were characterized by $^1$H NMR, $^{13}$C NMR, LCMS and HPLC.

Ethyl 2-(2-(3-(anthracen-10-yl)-2-(methoxymethoxy)-5-methylbenzylamino)-3-(4-hydroxyphenyl)propanamido) acetate (48a)
Yield : 86%

Nature : White solid.

mp : 189.7-189.9 °C

¹H NMR (DMSO-d₆/400 MHz); δ 8.65 (1H, s, H₂₂), 8.14 (3H, m, H₁, H₂₅), 7.55-7.51 (4H, m, H₂₆, H₂₇), 7.48-7.39 (2H, m, H₂₈), 7.27 (1H, s, H₆), 7.18 (2H, d, J=8.2 Hz, H₁₉), 6.97 (1H, s, H₈), 6.69 (2H, d, J=8.2 Hz, H₂₀), 4.10 (2H, s, H₁₂), 3.93-3.65 (6H, m, H₁₄, H₂₅, H₂₇), 3.35-3.33 (1H, m, H₁₆), 2.90 (1H, m, H₁₇), 2.58 (1H, m, H₁₇), 2.26 (6H, s, H₁₁, H₁₃), 1.28 (3H, t, H₂₈).

LCMS exhibited the molecular ion peak at EIMS m/z: 607.3 (M⁺+1).

UPLC purity; purity (97.87 %), method- ZX_1090MFAPVE.M, RT- 4.68 min.

Methyl 2-((S)-2-(3-(anthracen-10-yl)-2-(methoxymethoxy)-5-methylbenzylamino)-3-(4-hydroxyphenyl)propanamido)-4-methylpentanoate (48b)

Yield : 90%

Nature : Off-white solid

mp : 183.6-183.8 °C
LCMS exhibited the molecular ion peak at EIMS m/z: 579.3 (M^+1).

**UPLC purity;** purity (92.97 %), method- ZX_1090MFA.M, RT- 3.89 min.

(S)-2-(2-(3-(anthracen-10-yl)-2-(methoxymethoxy)-5-methylbenzylamino)-3-(4-hydroxy phenyl) propanamido)acetic acid (49a)

Yield : 85 %

Nature : Light brown solid.

mp : 187.3-188.9 °C

**^1H NMR spectral reports of 49a:** (DMSO-d_6/400 MHz); δ 9.32 (1H, brs, H_{22}), 8.63 (1H, brs, H_{24}), 8.12 (3H, m, H_1, H_{2a}), 7.53-7.47 (4H, m, H_{2d}, H_{2b}), 7.44-7.4 (2H, m, H_{2c}), 7.2 (1H, s, H_6), 7.05 (2H, d, J=8.2 Hz, H_{19}), 6.92 (1H, s, H_8), 6.66 (2H, d, J=8.2 Hz, H_{20}), 4.06-4.01 (2H, dd, J=5.6, 3.8 Hz, H_{12}), 3.81-3.65 (4H, m, H_{25}, H_{14}), 3.33-3.29 (1H, m, H_{16}), 2.86 (1H, m, H_{17}), 2.61 (1H, m, H_{17}), 2.49 (3H, s, H_{13}), 2.26 (3H, s, H_{11}).

**^13C NMR spectral reports of 49a:** (DMSO-d_6/100 MHz); δ 173.4 (C_{26}), 171.4 (C_{23}), 155.9 (C_{21}), 151.3, 133.7, 133.3, 133.2, 131.6, 130.9, 130.8, 130.2, 129.7, 128.5, 128.3, 126.6, 126.4, 126.3, 125.8, 125.3, 115, 98 (C_{12}), 63.4 (C_{16}), 55.4 (C_{13}), 46.1 (C_{25}), 40.1 (C_{14}), 38.8 (C_{17}), 20.4 (C_{11}).

**IR (KBr):** 3759 (O-H), 3276 (N-H), 2924 (C-H), 1700 (C=O), 1593(C=ONH), 1069 cm^{-1}.

LCMS exhibited the molecular ion peak at EIMS m/z: 579.3 (M^+1).

**UPLC purity;** purity (92.97 %), method- ZX_1090MFA.M, RT- 3.89 min.
$^1$H NMR spectral analysis of 49a: $^1$H NMR spectrum showed a singlet at $\delta$ 2.26 corresponds to methyl group (H$_{11}$) attached to the aromatic ring, peak at $\delta$ 2.49 corresponding to methyl group (H$_{13}$) in MOM protecting group (-OCH$_2$OCH$_3$), doublet of doublet at $\delta$ 2.61 due to (H$_{17}$) (-CH$_2$-) group attached to tyrosinyl amino acid, multiplet at $\delta$ 3.33-3.29 corresponds to methine group (H$_{16}$) in tyrosine amino acid moiety (N-CH-COOH), a doublet of doublet peak at $\delta$ 3.81-3.65 was observed as multiplet due to self coupling of germinal protons attached to (H$_{14}$, H$_{25}$) separately, a doublet of doublet peak at 4.06-4.01 for 2 protons due to germinal protons (H$_{12}$) whose coupling constant values are $J_{H_a,H_b}$=5.6 Hz, $J_{H_b,H_a}$=3.88 Hz) respectively, and a peak for H$_{20}$ was resonated as doublet at $\delta$ 6.66 with $J_{H19,H20}$=8.2 Hz, at $\delta$ 7.02 a single peak was seen due to H$_8$, a peak for H$_8$ was observed at $\delta$ 6.92 as singlet, due to coupling with H$_{20}$ proton, $\delta$ 7.05 doublet was observed due to (H$_{19}$) proton attached to the aromatic ring whose coupling constant value is $J$=8.2 Hz, singlet at $\delta$ 7.2 corresponds to phenyl proton (H$_6$), a multiplet was observed at $\delta$ 7.44-7.40 due to anthracenyl protons H$_{2c}$, $\delta$ 7.53-7.47 observed as multiplet for 4 protons corresponds to anthracenyl protons H$_{2d}$, H$_{2b}$, a multiplet peak at $\delta$ 8.12 for 3 protons corresponds to H$_1$, H$_{2a}$, a broad singlet peak at 8.63 due to amide NH proton, phenolic OH proton (H$_{24}$), and broad singlet peak at 9.32 due to phenolic OH proton.

$^{13}$C NMR spectral analysis of 49a: The spectrum displayed 28 signals; a signal at $\delta$ 20.4 (Ar-CH$_3$) due to methyl carbon attached to the phenyl ring, a signal at $\delta$ 38.8 corresponding to methylene carbon (C17) in tyrosine moiety CH$_2$-PHOH, a signal at $\delta$ 40.1 corresponding to benzyl(Ph-CH$_2$-NH) carbon C14, a signal at $\delta$ 46.1 (-NHCH$_2$COOH) due to glycine methylene group (C25) methyl carbon in MOM group, a singlet signal at $\delta$ 55.4 -OCH$_2$OCH$_3$ due to methyl carbon (C13) in MOM group, 63.4 (C16), a signal at 63.4 corresponds to (C16) methine carbon (N-CH-COOH) in tyrosine moiety, a signal at $\delta$ 98
(-OCH₂OCH₃) due to methylene carbon \((C12)\) in MOM protecting group, \((C20)\), a signal at \(\delta 115\) due to aromatic phenolic methylene carbon \((C20)\), a peak at \(\delta 125.3\) due to \((C9)\) which was coupling with \(C14\) carbon and signal at \(\delta 125.8\) corresponds to \((C5)\), similarly signals at 126.3, 126.4, 126.6, 128.3, 128.5, 129.7, 130.1, 130.2, 130.8, 130.9, were due to aromatic carbons, a peak at 131.6 corresponds to \(C7\) methyl attached phenyl carbon, a peak at 133.2 corresponds to \(C18\) tyrosinyl phenyl carbon, a peak for at 133.3 was corresponds to \(C4\) anthracene carbon which is attached to \(C5\) phenyl carbon, a signal at 133.7 is due to anthracene \(C_1\) carbon, a signal at 151.3 corresponds to \(C_{10}\) carbon which is attached to OMOM protecting group, a signal at 155.9 \((C_{21}-OH)\) is due to OH group attached phenolic carbon, a signal at 171.4 corresponds to \((C23)\) carbonyl carbon in amide group \((-CONH)\), a signal at 173.4 corresponds to \((C26)\) carbonyl carbon in acid group \((COOH)\).

**IR spectral analysis of 49a:**

The IR spectrum of 49a showed a sharp strong band at \(\nu = 3759 \text{ cm}^{-1}\) (OH stretching frequency of phenolic group), a strong single band at \(\nu = 3276 \text{ cm}^{-1}\) (NH stretching frequency of secondary amine), a strong band at \(\nu = 2924 \text{ cm}^{-1}\) (C-H stretching frequency of methyl group), a strong band at \(\nu = 2853 \text{ cm}^{-1}\) (O-H stretching frequency of carboxylic acid group), a strong band at \(\nu = 1700 \text{ cm}^{-1}\) (C=O stretching frequency of carboxylic acid group), a strong band at \(\nu = 1593 \text{ cm}^{-1}\) (C=O stretching frequency of amide group), a strong band at \(\nu = 1226 \text{ cm}^{-1}\) (C-O stretching frequency of both ether and phenolic group), a weak band at \(\nu = 1069 \text{ cm}^{-1}\) (C-N stretching frequency of alkyl amine).
2-((S)-2-(3-(Anthracen-10-yl)-2-(methoxymethoxy)-5-methylbenzylamino)-3-(4-hydroxy phenyl)propanamido)-4-methylpentanoic acid (49b)

![Chemical Structure of 49b]

Yield : 88 %
Nature : Off-white solid
mp : 170.3-171.4 °C

$^1$H NMR (400 MHz, DMSO-d$_6$): δ 9.17 (1H, brs, H$_{22}$), 8.65 (1H, s, NH), 8.20 (1H, d, H$_1$), 8.13 (2H, d, H$_{2a}$), 7.56-7.49 (4H, m, H$_{2d}$, H$_{2b}$), 7.45-7.40 (2H, m, H$_{2c}$), 7.2 (1H, s, H$_6$), 7.08-7.06 (2H, d, H$_{19}$), 6.94 (1H, s, H$_8$), 6.52 (2H, d, H$_{20}$), 4.35 (1H, m, H$_{25}$), 4.33-4.30 (1H, m, H$_{16}$), 4.11-4.06 (2H, dd, J = 8 Hz, J=16 Hz, H$_{12}$), 3.82-3.64 (2H, dd, J=12 Hz, H$_{14}$), 2.84 (1H, m, H$_{17}$), 2.65 (1H, m, H$_{17}$), 2.49 (3H, s, H$_{13}$), 2.28 (3H, s, H$_{11}$), 1.34 (3H, m, H$_{26}$, H$_{27}$), 0.85 (6H, m, H$_{28}$).

$^{13}$C NMR (100 MHz, DMSO-d$_6$): δ 174.6, 174.1, 156.2, 151.7, 134.3, 133.8, 133.5, 131.9, 131.3, 130.6, 130.4, 130.1, 129, 128.8, 127, 126.9, 126.7, 126.2, 125.6, 115.2, 98.4, 63.6 (C$_{16}$), 55.8 (C$_{14}$), 50.4 (C$_{26}$), 46.3 (C$_{17}$), 24.7 (C$_{11}$), 23.4 (C$_{28}$), 21.7 (C$_{28}$), 20.9 (C$_{27}$).

IR (KBr):3300(O-H), 3051(N-H), 2954 (C-H), 2868, 1650 (C=O), 1612 (CONH), 1514, 1445, 1225, 1154 cm$^{-1}$.

LCMS exhibited the molecular ion peak at EIMS m/z: 635.4 (M$^+$+1).

UPLC purity; purity (87.25 %), method- ZX_1090MFA.M, RT- 4.17 min.
Scheme-5

Synthesis of hydroxyl fluorescent amino acids

The MOM cleavage of 47 (a-d) was carried out using 4.5 M HCl in dioxane at room temperature for 5 h to afford 50 (a-d). All the compounds were characterized by $^1$H NMR, $^{13}$C NMR, LCMS and HPLC.

2-(3-(Anthracen-10-yl)-2-hydroxy-5-methylbenzylamino) acetic acid (50a)

Yield : 93 %

Nature : White solid

mp : 206.3-207.5 °C

$^1$H NMR spectral reports of 50a: (DMSO-d$_6$ / 300 MHz); δ 9.13 (2H, brs, H$_{12}$), 8.66 (1H, s, H$_1$), 8.14-8.12 (2H, m, J=8.37 Hz, H$_{2a}$), 7.54-7.47 (4H, m, H$_{2b}$, H$_{2d}$), 7.42-7.37 (3H, m, H$_{3}$H$_{2c}$), 6.98 (1H, d, H$_8$), 4.28 (2H, s, H$_{13}$), 3.90 (2H, s, H$_{15}$), 2.29 (3H, s, H$_{11}$).
$^{13}$C NMR spectral reports of 50a: (DMSO-$d_6$/100 MHz); δ 168.0 (C16), 151.7 (C10), 133.8 (C1), 132.5 (C4), 132.1 (C7), 131.1 (C8), 130.2, 128.4, 128.3, 126.7, 126.3, 125.7, 125.6, 125.1, 119.3 (C9), 46.5 (C15), 45.6 (C13), 20.0 (C11).

IR spectral reports of 50a: (KBr): 3553 (O-H-PhOH), 3345 (O-H from COOH), 2917, 2748, 1714 (C=O), 1419, 1314 cm$^{-1}$.

LCMS exhibited the molecular ion peak at EIMS m/z: 372.2 (M$^+$+1).

UPLC purity; purity (94.60 %), method- ZX_1090MFAPVE.M, RT- 4.250 min.

2-(3-(Anthracen-10-yl)-2-hydroxy-5-methylbenzylamino)propanoic acid (50b)

Yield : 91 %
Nature : Off-white solid
mp : 238.5 -239.5 °C

$^1$H NMR (DMSO-$d_6$/400 MHz); δ 9.67 (1H, brs, H$_{12}$), 9.32 (1H, brs, H$_{14}$), 8.67 (1H, s, H$_1$), 8.62 (1H, brs, H$_{18}$), 8.16-8.14 (2H, d, J=8.4 Hz,H$_{2a}$), 7.57-7.40 (7H, m, H$_{2b}$, H$_{2c}$, H$_{2d}$, H$_6$), 7.00-6.99 (1H, d, H$_8$), 4.3 (2H, d, H$_{13}$), 4.08 (1H, q, J=7.1 Hz,H$_{15}$), 2.32 (3H, s, H$_{11}$), 1.58 (3H, d, J=7.2 Hz, H$_{16}$).

$^{13}$C NMR (DMSO-$d_6$/100 MHz); δ 170.9 (C17), 151.6 (C10), 133.7 (C1), 132.6 (C4), 132.1, 131.2, 130.2, 128.4, 128.3, 126.7, 126.3, 125.9, 125.6 (C5), 125.1 (C9), 119.7, 64.90, 54.47 (C15), 44.25 (C13), 20.02 (C11), 14.69 (C16).
IR (KBr): 3392 (O-H-PhOH), 2803, 1746 (C=O), 1435, 1200 cm⁻¹.

LCMS exhibited the molecular ion peak at EIMS m/z: 386.2 (M⁺+1).

UPLC purity; purity (97.87 %), method- ZX_1090MFAPVE.M, RT- 4.68 min.

2-(3-(Anthracen-10-yl)-2-hydroxy-5-methylbenzylamino)-3-(4-hydroxyphenyl) propanoic acid (50c)

Yield : 91 %

Nature : Yellowish solid

mp : 217.3-218.7°C

¹H NMR (DMSO-d₆/400 MHz); δ 9.32 (2H, brs, H₁₂), 8.62 (1H, s, H₁), 8.13 (2H, d, J=8 Hz, H₂a), 7.60-7.56 (2H, m, H₂b), 7.53-7.48 (2H, m, H₂c), 7.04-6.88 (4H, m, H₆, H₈, H₁₈), 6.61 (2H, d, H₁₉), 4.03 (1H, s, J=12 Hz, H₁₆), 3.85 (1H, s, J=16 Hz, H₁₆), 3.40 (1H, t, J=4 Hz, H₁₅), 2.91-2.86 (1H, m, H₁₆), 2.80-2.73 (1H, m, H₁₆), 2.26 (3H, s, H₁₁).

¹³C NMR (DMSO-d₆/100 MHz); δ 174.9 (C₂₂), 156.3 (C₂₀), 149.3 (C₁₀), 132.2 (C₁), 131.5 (C₄), 130.5 (C₁₇), 130.4, 130.3, 130.2, 128.8, 128.7, 127.9, 127.1, 127.0, 126.4, 125.7, 125.6, 125.2 (C₉), 115.5 (C₁₉), 62.5 (C₁₅), 49.4 (C₁₃), 37.4 (C₁₆), 20.5 (C₁₁).

IR (KBr): 3504 (O-H-PhOH), 3189 (O-H), 2922 (N-H), 1613 (C=O), 1515, 1434, 734 cm⁻¹.

LCMS exhibited the molecular ion peak at EIMS m/z: 478.2 (M⁺+1).

UPLC purity; purity (99.09 %), method- ZX_1090MFAPVE.M, RT- 4.219 min.
2-(3-(Anthracen-10-yl)-2-hydroxy-5-methylbenzylamino)-3-hydroxypropanoic acid (50d)

Yield : 88 %

Nature : yellowish solid

mp : 178.2-179.4°C

$^1$H NMR (DMSO-d$_6$/400 MHz); $\delta$ 9.2 (2H, brs, H$_{12}$), 8.64 (1H, s, H$_1$), 8.13 (2H, d, J=8.3 Hz, H$_{2a}$), 7.56-7.47 (4H, m, H$_{2d}$, H$_{2b}$), 7.41-7.33 (3H, m, H$_6$, H$_{2c}$), 6.95 (1H, s, H$_8$), 4.24 (2H, m, H$_{16}$), 3.87-3.77 (2H, m, H$_{13}$), 3.15 (1H, s, H$_{15}$), 2.28 (3H, s, H$_{11}$).

$^{13}$C NMR (DMSO-d$_6$/100 MHz); $\delta$ 169.8 (C$^{18}$), 152.7 (C$^{10}$), 133.7 (C$^1$), 133.4 (C$^4$), 132.2, 131.6, 130.6, 128.8, 128.4, 127.1, 126.8, 126, 125.9, 125.6 (C$^9$), 61.7 (C$^{15}$), 59.9 (C$^{16}$), 46.7 (C$^{13}$), 20.5 (C$^{11}$).

IR (KBr): 3232 (O-H-PhOH), 1717, 1617(C=O), 1445, 1216, 1033, 734, 513 cm$^{-1}$.

LCMS exhibited the molecular ion peak at EIMS $m/z$: 402.2 (M$^+$+1).

UPLC purity; purity (98.13 %), method- ZX_1090MFAPVE.M, RT- 4.165 min.
CHAPTER-2

Discussion of carbazolyl fluorescent sensors

Synthesis of 3- (9H-carbazol-9-yl)-2-(methoxymethoxy)-5-methylbenzaldehyde (57)

The core moiety for the synthesis of fluorescent amino acid and peptide was carbazolyl aldehyde, since it has fluorescent property. It was prepared by 6 step synthesis. First step was the protection of phenolic group (51) in 2-bromo-4-methyl phenol by using ethoxy methyl chloride, diisopropyl ethyl amine as a base and dichloromethane as a solvent for 4 h at room temperature with 90% good yield. Introduction of carbazole moiety in 2-position was done by substitution of bromo group in 51 by Buckwald condition. It was carried out by refluxing a mixture of carbazole (52), trans-1,2 diamino cyclohexane, potassium phosphate, copper iodide for 8 h at 110 °C to get intermediate 53 as a pure solid after column purification.

Scheme-6

Synthesis of 3- (9H-Carbazol-9-yl)-2-(methoxymethoxy)-5-methylbenzaldehyde
Direct insertion of aldehyde functionality in 2\textsuperscript{nd} position was tried in intermediate 53. It was carried out by treating 53 with n-BuLi in THF followed by DMF quenching, it gave poor yields. Another attempts was also tried by deprotection of ethoxy methyl group in 53 followed by in situ protection of phenolic group by adding sodium hydride in tetrahydrofuran followed by addition of n-butyl lithium for anion generation at 2\textsuperscript{nd} position and DMF as a formylating agent. This method also gave very less yield with many spots in TLC.

With a view to improving the yield of the reaction, insertion of bromo group in 2\textsuperscript{nd} position was necessary for formylation in intermediate 53. It was carried out by deprotection of EOM group by using 4.5 M HCl in dioxane at room temperature for 5 h followed by bromination by using N-bromo succinimide, triethyl amine and DCM as solvent at room temperature to afford the required intermediate 55 as white solid. Before formylation step, protection of phenolic group was achieved by treating MOMCl, diisopropyl ethyl amine and dichloromethane for 4 h at room temperature. Formylation of bromo intermediate 56 was carried out by using n-BuLi and THF at -78 °C for 3 h (anion generation) followed by addition of dimethyl formamide to afford 3-((9H-Carbazol-9-yl)-2-(methoxymethoxy)-5-methylbenzaldehyde (57) as yellow semi-solid.

2-Bromo-1-(ethoxymethoxy)-4-methylbenzene (51)

![Chemical Structure](image)

yield : 98 %

Nature : Brownish liquid
$^1$H NMR (CDCl$_3$/300 MHz); δ 7.36 (1H, s, H-Ph), 7.08-7.05(2H, m, H-Ph), 5.26 (2H, s, OCH$_2$), 3.81-3.74(2H, q, J=7.08 Hz, CH$_2$CH$_3$), 2.27 (s, 3H, C-CH$_3$), 1.24-1.20 (3H, t, CH$_2$CH$_3$).

$^{13}$C NMR (CDCl$_3$/75 MHz); δ 145.82 (C-O), 133.60 (C-CH$_3$), 132.44 (C7), 126.90 (C10), 116.88 (C8), 112.42 (C-Br), 96.72 (OCH$_2$O), 62.95 (OCH$_2$CH$_3$), 22.63 (C-CH$_3$), 14.20 (OCH$_2$CH$_3$) ppm.

9-(2-(Ethoxymethoxy)-5-methylphenyl)-9H-carbazole (53)

Yield : 98 %

Nature : White solid.

$^1$H NMR (DMSO -d$_6$/300MHz); δ 8.16-8.13 (2H, d, J=7.68 Hz, H$_2$). 7.41(2H, m, Cbz-H), 7.39-7.24 (3H, m, Cbz-H, H$_8$), 7.20-7.17 (2H, d, H$_{10}$, H$_{11}$), 4.96 (2H, s, H$_{14}$), 3.39-3.32 (2H, q, J=7.05 Hz,H$_{15}$), 2.39 (3H, s, H$_{13}$), 1.01 (3H, t, J=7.08 Hz, H$_{16}$) ppm.

NMR (DMSO -d$_6$/75 MHz); δ 144.66 (C12), 140.29 (C6), 132.60 (C9), 132.32 (C7), 127.49 (C10), 125.99 (C3), 125.22 (C1), 123.96 (C4), 119.96 (C2), 118.88 (C8), 114.81 (C11), 111.68 (C5), 97.47 (C14), 63.72 (C15), 22.93 (C13), 14.20 (C16) ppm.

LCMS exhibited the molecular ion peak at EIMS m/z: 330.4 (M$^+$-1)
2-(9H-Carbazol-9-yl)-4-methylphenol (54)

Yield : 88 %

Nature : Viscous liquid

$^1$H NMR (CDCl$_3$/300 MHz); $\delta$ 8.17-8.15 (2H, d, J=7.68 Hz, H$_2$), 7.47-7.46 (2H, m, H$_5$), 7.35-7.14 (5H, m, H$_3$, H$_4$, H$_8$), 7.13-7.11 (2H, d, H$_{10}$, H$_{11}$), 4.88 (1H, s, OH), 2.35 (3H, s, H$_{13}$) ppm.

NMR (CDCl$_3$/75 MHz); $\delta$ 147.69 (C12), 140.32 (C6), 132.63 (C9), 133.18 (C7), 127.84 (C10), 126.38 (C3), 126.12 (C1), 123.66 (C4), 121.26 (C2), 119.34 (C8), 117.84 (C11), 110.98 (C5), 22.71 (C13) ppm.

UPLC exhibited the molecular ion peak at EIMS m/z: 274.4 (M$^+$+1).

2-Bromo-6-(9H-carbazol-9-yl)-4-methylphenol (55)

Yield : 90 %

Nature : White solid

$^1$H NMR (CDCl$_3$/300 MHz); $\delta$ 8.16-8.14 (2H, d, J=7.65 Hz, H$_2$), 7.50-7.40 (3H, m, H$_5$, H$_{10}$), 7.34-7.25 (2H, m, H$_4$), 7.20-7.17 (3H, m, H$_3$, H$_8$), 5.41(1H, s, OH), 2.36 (3H, s, H$_{13}$) ppm.
\(^{13}\text{C NMR}\) (CDCl\textsubscript{3}/75 MHz);  \(\delta\) 151.79 (C12), 140.42 (C6), 134.83 (C9), 135.16 (C7), 132.94 (C10), 127.99 (C3), 126.51 (C1), 123.57 (C4), 121.38 (C2), 118.94 (C8), 115.62 (C11), 111.26 (C5), 22.43 (C13) ppm.

\textbf{UPLC} exhibited the molecular ion peak at EIMS \(m/z: 352.3\) (M\textsuperscript{+}+1).

\textit{9-(3-Bromo-2-(methoxymethoxy)-5-methylphenyl)-9H-carbazole (56)}

![Structure of 56]

Yield : 96%

Nature : yellowish soild

\(^1\text{H NMR}\) (CDCl\textsubscript{3}/300 MHz);  \(\delta\) 8.14-8.11 (2H, d,  \(J=7.74\) Hz, \(\text{H}_2\)), 7.58 (1H, d, \(\text{H}_{10}\)), 7.46-7.41(2H, m, \(\text{H}_5\)), 7.33-7.25 (5H, m, \(\text{H}_3, \text{H}_4, \text{H}_8\)), 4.52 (2H, s, \(\text{H}_{14}\)), 2.67 (3H, s, \(\text{H}_{15}\)), 2.39 (3H, s, \(\text{H}_{13}\)) ppm.

\(^{13}\text{C NMR}\) (CDCl\textsubscript{3}/75 MHz);  \(\delta\) 153.94 (C12), 140.12 (C6), 134.76 (C9), 133.46 (C7), 132.17 (C10), 128.77 (C3), 126.48 (C1), 123.52 (C4), 120.32 (C2), 118.34 (C8), 113.99 (C11), 110.89 (C5), 97.78 (C14), 54.48 (C15), 22.23 (C13) ppm.

\textbf{LCMS} exhibited the molecular ion peak at EIMS \(m/z: 396.1\) (M\textsuperscript{+}+1).

\textit{3-(9H-Carbazol-9-yl)-2-(methoxymethoxy)-5-methylbenzaldehyde (57)}

![Structure of 57]

Yield : 84%
Nature: Yellow semi solid.

\(^1\)H NMR (CDCl\(_3/400\) MHz); \(\delta\) 10.52 (1H, s, H\(_{17}\)), 8.13-8.15 (2H, d, \(J=7.74\) Hz, H\(_2\)), 7.84 (1H, d, H\(_{16}\)), 7.55 (1H, d, H\(_8\)), 7.41-7.46 (2H, m, H\(_3\), H\(_4\)), 4.40 (2H, s, H\(_{14}\)), 2.95 (3H, s, H\(_{15}\)), 2.45 (3H, s, H\(_{13}\)) ppm.

\(^{13}\)C NMR (CDCl\(_3/100\) MHz); \(\delta\) 185.82 (C\(_{17}\)), 151.82 (C\(_{12}\)), 140.52 (C\(_6\)), 132.89 (C\(_9\)), 132.89 (C\(_7\)), 129.91 (C\(_{11}\)), 129.08 (C\(_{10}\)), 128.87 (C\(_3\)), 126.58 (C\(_1\)), 124.22 (C\(_8\)), 123.16 (C\(_4\)), 121.25 (C\(_2\)), 111.01 (C\(_5\)), 98.90 (C\(_{14}\)), 55.68 (C\(_{15}\)), 23.01 (C\(_{13}\)) ppm.

UPLC exhibited the molecular ion peak at EIMS \(m/z\): 316.1 (M\(^+\)-1). (aldehyde moiety cleaved)

**Scheme-7**

**Synthesis of carbozole fluorescent amino acids (59a-b)**

Synthesis of carbozole fluorescent amino acid was attempted by treating carbozole core amino acid with different amino esters (glycinyl, L-tyrosynyl esters) by reductive amination method, and the resulting carbozolyl esters were hydrolysed by using mixture of solvents with Lithium hydroxide at room temperature for 5 h. All the new compounds were thoroughly characterised by analytical and spectral methods, like \(^1\)H NMR, \(^{13}\)C NMR, mass spectroscopy and melting points.
Ethyl 2-(3-(9H-carbazol-9-yl)-2-(methoxymethoxy)-5-methylbenzylamino) acetate (58a)

Yield : 97.7 %
Nature : Viscous yellow oil
mp : 189.1-190.3 °C

$^1$H NMR (CDCl$_3$/300 MHz); δ 8.14-8.11 (2H, d, H$_2$), 7.45-7.39 (2H, m, H$_3$), 7.34-7.23 (5H, m, H$_3$, H$_4$, H$_{10}$), 7.18 (1H, d, H$_8$), 4.29 (2H, s, H$_{14}$), 4.25-4.18 (2H, m, J=7.11Hz, H$_{20}$), 3.97 (2H, s, H$_{16}$), 3.52 (2H, s, H$_{18}$), 2.88 (3H, s, H$_{15}$), 2.38 (3H, s, H$_{13}$), 1.29 (3H, t, J=7.11 Hz, H$_{21}$) ppm.

$^{13}$C NMR (CD$_3$OD) /100 MHz); δ 173.22 (C19), 151.71 (C12), 142 (C6), 136.38 (C9), 135.22 (C10), 132.17 (C7), 131.07 (C11), 130.33 (C3), 127.05 (C4, C1), 124.62 (C8), 121.04 (C2), 111.37 (C5), 100.25 (C14), 61.90 (C20), 57.31 (C15), 50.35 (C18), 49 (C16), 20.77 (C13), 14.55 (C21) ppm.

LCMS exhibited the molecular ion peak at EIMS $m/z$: 434 (M$^+$+1).

(S)-Ethyl 2-(3-(9H-carbazol-9-yl)-2-(methoxymethoxy)-5-methylbenzylamino)-3-(4-hydroxyphenyl)propanoate (58b)

Yield : 92 %
Nature: Viscous yellow oil

mp: 202.3-203.5 °C

$^1$H NMR (DMSO-d$_6$/300 MHz); δ 9.18 (1H, s, H$_{24}$), 8.21-8.18 (2H, d, H$_2$), 7.42-7.37 (2H, m, H$_5$), 7.27-7.10 (6H, m, H$_3$, H$_4$, H$_{31}$), 7.01-6.92 (2H, m, H$_8$, H$_{10}$), 6.66-6.61(2H, m, H$_{22}$), 4.18 (2H, s, H$_{14}$), 4.0 (2H, q, H$_{26}$), 3.76 (1H, m, H$_{18}$), 3.46 (2H, s, H$_{16}$), 2.70 (2H, m, H$_{19}$), 2.36 (3H, s, H$_{15}$), 2.29 (3H, s, H$_{13}$), 1.18 (3H, t, H$_{27}$) ppm.

$^{13}$C NMR (CD$_3$OD/100 MHz); δ 172.22 (C$_{25}$), 151.71 (C$_{23}$), 142 (C$_{12}$), 136.38 (C$_6$), 135.22 (C$_{20}$), 132.17, 131.07, 130.33, 127.05, 124.62, 121.04, 111.37, 100.25 (C$_{14}$), 61.90 (C$_{26}$), 57.31 (C$_{18}$), 50.35 (C$_{15}$), 49 (C$_{16}$), 48.75 (C$_{19}$), 20.77 C$_{13}$) 14.55 (C$_{27}$) ppm.

LCMS exhibited the molecular ion peak at EIMS $m/z$: 539.2 (M$^+$+1).

2-(3-(9H-Carbazol-9-yl)-2-(methoxymethoxy)-5-methylbenzylamino) acetic acid (59a)

![59a](image)

Yield: 96 %

Nature: Off-white solid

mp: 191.2-192.5 °C

$^1$H NMR (DMSO-d$_6$/400 MHz); δ 8.25-8.23 (2H, d, H$_2$), 7.44-7.41 (2H, m, H$_5$), 7.28-7.26 (4H, m, H$_{3, 4}$), 7.21-7.19 (2H, m, H$_8$, H$_{10}$), 4.76 (2H, s, H$_{17}$), 4.26 (2H, s, H$_{14}$), 4.18 (2H, s, H$_{16}$), 3.51 (2H, s, H$_{18}$), 2.74 (3H, s, H$_{15}$), 2.35 (3H, s, H$_{13}$) ppm.

$^{13}$C NMR (DMSO-d$_6$/75 MHz); δ 164.88 (C$_{19}$), 149.87 (C$_{12}$), 140.57 (C$_6$), 135.46 (C$_9$), 131.51 (C$_7$), 129.76 (C$_{10}$), 129.71 (C$_{11}$), 129.44 (C$_3$), 126.63 (C$_1$), 123.12 (C$_4$), 120.83
(C2), 120.41 (C8), 110.66 (C5), 99.04 (C14), 56.88 (C15), 50.18 (C18), 44.17 (C16), 20.81 (C13) ppm.

**LCMS** exhibited the molecular ion peak at EIMS m/z: 403.5 (M⁺-1).

**(S)-2-(3-(9H-carbazol-9-yl)-2-(methoxymethoxy)-5-methylbenzylamino)-3-(4-hydroxyphenyl)propanoic acid (59b)**

![Chemical Structure of 59b](image)

Yield: 89 %

Nature: off-white solid

mp: 213.7-214.3 °C

**1H NMR** (DMSO-d$_6$/400 MHz); δ 9.22 (1H, brs, H$_{24}$), 8.22-8.20 (2H, d, H$_2$), 7.42-7.41(2H, m, H$_5$), 7.28-7.11 (6H, m, H$_{3,4,21}$), 7.07-7.05 (2H, d, H$_{8,10}$), 6.68-6.66 (2H, d, H$_{22}$), 4.19 (2H, d, H$_{14}$), 3.73-3.89 (2H, m, H$_{16}$), 3.39 (1H, t, H$_{18}$), 2.88-2.92 (1H, m, H$_{19}$), 2.73-2.76 (1H, m, H$_{19}$), 2.71 (3H, s, H$_{15}$), 2.31 (3H, s, H$_{13}$) ppm.

**13C NMR** (CD$_3$OD/100 MHz); δ 175.12 (C25), 151.84 (C23), 142.56 (C12), 136.49 (C6), 136.12 (C20), 133.37, 131.19, 131.13, 127.83, 125.22, 122.54, 111.42, 100.48 (C14), 59.11(C18), 50.55 (C15), 49.13 (C16), 49.36 (C19), 20.83 (C13) ppm.

**LCMS** exhibited the molecular ion peak at EIMS m/z: 511.2 (M⁺+1).
Scheme-8

Synthesis of carbazole peptide derivatives

The carbazole peptide derivatives (62a-b) were synthesised by reacting carbazole -L-tyrosinyl amino acid derivative with different racemic amino esters (glycinyl, valine) using EDC in acetonitrile as a solvent at room temperature for 16 h to afford 61(a-b), followed by hydrolysis of the esters using lithium hydroxide at room temperature with good yield. All the new compounds were thoroughly characterised by analytical and spectral methods, like $^1$H NMR, $^{13}$C NMR, mass spectroscopy and melting points.

(S)-methyl 2-(2-(3-(9H-carbazol-9-yl)-2-(methoxymethoxy)-5-methylbenzylamino)-3-(4-hydroxyphenyl)propanamido)acetate (61a)

Yield : 98.13 %

Nature : Off-white solid
mp: 210.4-211.7 °C

\[^1\text{H} \text{NMR}\] (DMSO-\text{d}_6/400 \text{ MHz}); \delta 9.26 (1H, brs, H_{24}), 8.20-8.18 (2H, d, H_2), 7.40-7.42 (2H, m, H_5), 7.33-7.17 (6H, m, H_3, H_4, H_{21}), 7.14-7.19 (2H, d, H_8, H_{10}), 6.73-6.71 (2H, d, H_{22}), 4.21 (2H, d, H_{14}), 4.21 (2H, d, H_{26}), 3.61 (3H, s, H_{28}), 3.71-3.88 (2H, m, H_{16}), 3.33 (1H, t, H_{18}), 2.87-2.89 (1H, m, H_{19}), 2.69-2.72 (1H, m, H_{19}), 2.72 (3H, s, H_{13}), 2.34 (3H, s, H_{13}) ppm.

LCMS exhibited the molecular ion peak at EIMS m/z: 581.6 (M^+1).

**Methyl 2-((S)-2-(3-(9H-carbazol-9-yl)-2-(methoxymethoxy)-5-methylbenzylamino)-3-(4-hydroxyphenyl)propanamido)-4-methylpentanoate (61b)**

![Methyl 2-((S)-2-(3-(9H-carbazol-9-yl)-2-(methoxymethoxy)-5-methylbenzylamino)-3-(4-hydroxyphenyl)propanamido)-4-methylpentanoate (61b)](image)

Yield: 92.4 %

Nature: White solid

mp: 199.5-200.1 °C

\[^1\text{H} \text{NMR}\] (DMSO-\text{d}_6/400 \text{ MHz}); \delta 9.1 (1H, brs, OH), 7.52-7.34 (2H, m, H_2), 7.24-7.18 (2H, m, H_5), 7.09-6.94 (8H, m, H_3, H_4, H_8, H_{10}, H_{21}), 6.54 (2H, d, H_{22}), 4.08 (2H, s, H_{14}), 3.78-3.60 (2H, dd, H_{16}), 3.60 (3H, s, H_{31}), 3.22 (1H, m, H_{26}), 2.76 (1H, m, H_{19}), 2.58 (1H, m, H_{19}), 2.49 (3H, s, H_{15}), 2.35 (1H, d, H_{18}), 2.20 (3H, s, H_{13}), 1.59 (1H, m, H_{28}), 1.49 (2H, m, H_{27}), 0.88 (6H, d, H_{29}) ppm.

LCMS exhibited the molecular ion peak at EIMS m/z: 638.4 (M^+1).

(S)-2-((S)-2-(3-(9H-carbazol-9-yl)-2-(methoxymethoxy)-5-methylbenzylamino)-3-(4-hydroxyphenyl)propanamido)acetic acid (62a)
Yield: 89.55%

Nature: White solid

mp: 217.5-218.3 °C

$^1$H NMR (DMSO-d$_6$/400 MHz); δ 9.27 (1H, brs, H$_{24}$), 8.20-8.18 (2H, d, H$_2$), 7.38-7.39 (2H, m, H$_5$), 7.30-7.15 (6H, m, H$_{3,4,21}$), 7.11-7.20 (2H, d, H$_{8,10}$), 6.70-6.68 (2H, d, H$_{22}$), 4.22 (2H, d, H$_{14}$), 4.20 (2H, d, H$_{26}$), 3.75-3.91 (2H, m, H$_{16}$), 3.35 (1H, t, H$_{18}$), 2.86-2.88 (1H, m, H$_{19}$), 2.70-2.73 (1H, m, H$_{19}$), 2.73 (3H, s, H$_{13}$), 2.32 (3H, s, H$_{13}$) ppm.

LCMS exhibited the molecular ion peak at EIMS m/z: 568.3 ($M^+$+1).

2-((S)-2-(3-(9H-carbazol-9-yl)-2-(methoxymethoxy)-5-methylbenzylamino)-3-(4-hydroxyphenyl)propanamido)-4-methylpentanoic acid (62b)

Yield: 96.43%

Nature: Pluffy yellow solid

mp: 211.2-212.8 °C
$^1$H NMR (DMSO-d$_6$/400 MHz); δ 9.3 (1H, brs, OH), 8.17 (3H, m, CONH, H$_2$), 7.42-7.40 (2H, m, H$_5$), 7.38-7.06 (11H, m, H$_3$, H$_4$, H$_8$, H$_{10}$, H$_{21}$, NH), 6.69 (2H, d, J=8.36 Hz, H$_{22}$), 4.14 (2H, s, H$_{14}$), 3.81-3.65 (2H, dd, J=14.36 Hz, H$_{16}$), 3.28 (1H, m, H$_2$), 2.83 (1H, m, H$_{19}$), 2.63 (1H, m, H$_{19}$), 2.50 (3H, s, H$_{15}$), 2.37 (1H, d, J=1.8 Hz, H$_{18}$), 2.25 (3H, s, H$_{13}$), 1.55 (1H, m, H$_{28}$), 1.54 (2H, m, J=8.68, 4.16 Hz, H$_{27}$), 0.86 (6H, d, J=6.56 Hz H$_{29}$) ppm.

$^{13}$C NMR (DMSO-d$_6$/100 MHz); δ 174.82 (C$_{30}$), 173.98 (C$_{25}$), 156.26 (C$_{23}$), 149.61(C$_{12}$), 140.67 (C$_6$), 135.69 (C$_{20}$), 134.70 (C$_9$), 130.66 (C$_{21}$), 129.41, 129.03, 128.18, 126.46, 125.70, 123.01, 120.68, 120.19, 115.29, 110.78, 110.60, 98.8 (C$_{14}$), 63.51 (C$_{18}$), 56.32 (C$_{15}$), 50.61 (C$_{26}$), 46.10 (C$_{16}$), 40.9 (C$_{27}$), 39.10 (C$_{19}$), 24.82 (C$_{13}$), 23.46 (C$_{29}$), 21.78 (C$_{29}$), 20.80 (C$_{28}$) ppm.

IR (KBr): 3776 (O-H), 3051 (N-H), 2924 (C-H), 1720 (C=O), 1593 (C=ONH), 1514 cm$^{-1}$.

LCMS exhibited the molecular ion peak at EIMSm/z: 624.4 (M$^+$+1).

$^1$H NMR spectral analysis of 62b:

$^1$H NMR spectrum showed a singlet at δ 0.86 corresponds to dimethyl group (6H, H$_{29}$) attached to leucine group, peak at δ 1.54 was observed as multiplet by coupling with H$_{26}$ and H$_{28}$ protons which corresponds to methylene group (H$_{27}$) attached to leucine, a multiplet peak was observed δ 1.55 corresponds to methane proton attached to isopropyl group which coupled with H$_{27}$ and H$_{29}$ protons, at δ 2.25 a singlet peak was observed which corresponds to methyl group (H$_{13}$) proton attached to the aromatic ring, a doublet peak at δ 2.37 was corresponds to methine proton attached to L-tyrosine group by coupling with (H$_{18}$) proton, a singlet peak at δ 2.50 was corresponds to methyl proton (H$_{15}$) from MOM group, at δ 2.63 and 2.83, multiplet peak (gem-coupling) was observed which corresponds to methylene protons (H$_{19}$) in L-tyrosine group, a multiplet peak at δ 3.28 was corresponds to methylene protons (H$_{26}$), a doublet of doublet at δ 3.81-3.65 due to (H$_{16}$), J=14.36 Hz), singlet at δ 4.14
corresponds to methylene group (H_{14}) proton, (Ar-CH_{2}-NH), a δ 6.69 doublet was due to (H_{22}) tyrosine aromatic protons, Peak between 7.42-7.06 due to 11 aromatic protons (H_{3}, H_{4}, H_{5}, H_{8}, H_{10}, H_{21}, NH), and a peak for 3 protons (H_{2}, amide proton) was resonated as multiplet at δ 8.17, at δ 9.3 as broad singlet was observed due to (OH) proton attached to the tyrosine ring.

^{13}C NMR spectral analysis of 62b: The spectrum displayed 30 signals; a signal at δ 20.8 was due to C28 methine carbon in leucine, a signal at δ 23.4 and 21.7 was due to C29 carbon (dimethyl group in leucine), a signal at δ 24.8 (Ar-CH_{3}) due to methyl carbon attached to the phenyl ring (C13), a signal at δ 39.1 corresponding to methylene carbon (C19) in tyrosine moiety CH_{2}-PHOH, a signal at δ 40.9 corresponding C26 carbon, a signal at δ 46.1 corresponding to benzyl (Ph-CH_{2}-NH) carbon C16, δ 50.6 was due to methine carbon (C26) in leucine group, δ 56.3 -OCH_{2}OCH_{3}) due to methyl carbon (C15) in MOM group, a peak at δ 63.5 due to (C18) signal, at δ 98.8 corresponds to (C14), similarly signals at 129.4, 129.0, 128.1, 126.4, 125.7, 123.0, 120.6, 120.1, 115.2, 110.7 110.6 were due to aromatic carbons, a peak at 130.6 corresponds to (C21) tyrosine ring carbon, a peak at 134.7 corresponds to C9 phenyl carbon, a peak for at 135.6 was corresponds to C20 in tyrosine group, a signal at 140.6 is due to carbazole C_{6} carbon, a signal at 149.6 corresponds to C_{12} carbon which is attached to OMOM protecting group ,a signal at 156.2 (C_{23}-OH) is due to OH group attached phenolic carbon, a signal at 173.9 corresponds to (C25) carbonyl carbon in amide group (-CONH), a signal at 174.82 corresponds to (C30) carbonyl carbon in acid group (COOH).

IR spectral analysis of 62b:

The IR spectrum of 62b showed a sharp strong band at ν = 3776 cm\(^{-1}\) (OH stretching frequency of phenolic group), a strong band at ν=3051 cm\(^{-1}\) (NH stretching frequency of
secondary amine), a strong band at $\nu=2924$ cm$^{-1}$ (C-H stretching frequency of methyl group), a strong band at $\nu=1720$ cm$^{-1}$ (C=O stretching frequency of carboxylic acid group), a strong band at $\nu=1593$ cm$^{-1}$ (C=O stretching frequency of amide group).

Compound 62b showed the molecular ion peak at 624.4 (M$^+$+1) in LCMS consistent with the molecular formula C$_{37}$H$_{41}$N$_3$O$_6$. 
FLUORESCENCE STUDIES

Absorption and fluorescence spectra

Fluorescence PET sensors, in general, have been developed as pH sensors or cation sensors for detecting cations such as H\(^+\), Na\(^+\), K\(^+\), Ca\(^{2+}\), Mg\(^{2+}\), Cu\(^{2+}\), Zn\(^{2+}\), Pb\(^{2+}\), and Cd\(^{2+}\) as well as neutral organic species such as saccharides in biochemical analyses. They composed of a fluorophore skeleton linked to a cation binding site such as an amino moiety via a methylene spacer (fluorophore-spacer-receptor structure). The anthracene amino acids synthesised were evaluated for their UV absorption and fluorescence emission spectrometry (Figure-17) similar to the procedures available in literature\(^1\)\(^3\).

![Fluorophore and Receptor binding site](image)

**Figure-16**

![UV absorption and Fluorescence spectra](image)

**Figure-17**

In UV absorption spectrum the characteristic anthracene absorption and emission were observed with corresponding excitation wavelength. The anthracene
amino acid 47c was tested for metal ion complexation with various metal ion solutions. 24 µM concentration of the fluorescent amino acid was able to detect sub-micro molar concentrations of iron metal ions more specifically and efficiently.

With the applied excitation wavelength 365 nm, the emission was observed at 415 nm. At this emission value the metal ion quenching effect of the fluorescent amino acid was studied. Though all the tested metal ions (Figure-18) showed interaction with the amino acid at 24 µM concentration, iron showed enhanced activity by means quenching the fluorescence of 47c.

![Figure-18](image)

Figure-18

Among Co²⁺, In³⁺, Fe³⁺, Mn²⁺, Ba²⁺, Zn²⁺ ions tested, at ~10µM concentration Fe³⁺ completely quenched the fluorescence of 47c in comparison with approximately similar concentration of the other metal ions (Figure-18). Even 0.3 µM concentration of Fe³⁺ was detected by the fluorescent amino acid and at 10µM concentration there was a complete quenching by Fe³⁺ ion. With this objective to detect hazardous metal ion contamination in a particular polluted or test sample by means of fluorescence we have synthesised these fluorescent amino acids that could detect iron (III) ion preferentially and efficiently up to sub-micro molar concentrations. The tentative image of complex formed (Figure-19) is depicted below.
Upon addition of Cobalt ion (Co\(^{2+}\)) in the concentration range (4.2 µM to 840 µM) there was significant quenching at 120 µM. During the initial 8 µM, 12µM addition of metal ions there was a slight enhancement of emission and further addition of metal ions resulted in quenching of the anthracene based amino acid fluorescence emission. Indium metal ion in the concentration range (3.33 µM to 660 µM) was used and there was enhancement of emission. Fe\(^{3+}\) metal ion in the concentration range (0.37 µM to 74 µM) was used to study the interaction and with 3.7 µM significant binding was noted whereas ~10 µM completely quenched the fluorescence of the amino acid. Mn\(^{2+}\) with concentration (4.08 µM to 800 µM), Ba\(^{2+}\) with concentration (3.9 µM to 780 µM), Zn\(^{2+}\) with concentration (3.37 µM to 634 µM), Ni\(^{2+}\) with concentration (3.45 µM to 690 µM) and La\(^{3+}\) with concentration (2.31 µM to 460 µM) were also tested. In all the metal ions except Ni\(^{2+}\), there was enhancement of emission which was due to the metal ion itself and the emission increased with the increase in metal ion concentration. In the case of Nickel metal ion there was significant quenching effect at 34 µM concentration of the metal ion. Thus the fluorescent tyrosine amino acid quenches the metal ions Fe\(^{3+}\), Co\(^{2+}\), and Ni\(^{2+}\) but the quenching effect was very significant in the case of Fe\(^{3+}\) and the quenching was more selective (Figure-20).
Pathogen Bio-sensing

The fluorescent amino acids were subjected to fluorescently sense and mark the pathogens/normal cells. Anthracene and carbazole based fluorescent amino acid was synthesized, purified and characterized by $^1$H-NMR, and Mass spectral analysis. Both the fluorescent amino acids were found to be helpful for fluorescently marking breast cancer MCF7 cells being imaged. The chosen compounds exhibited mild antibacterial activity against Gram +ve and Gram –ve bacteria. The carbazole and anthracene amino acids helped to mark the cells with green and blue fluorescence.

The tested carbazole amino acid showed better cytocompatibility than the anthracene amino acids. Toxicity of anthracene biosensor was more as compared to carbazole sensor and so, we chose to proceed with carbazole as a biomarker.

Biocompatibility

The biocompatibility evaluation of the fluorescent amino acids as biomarkers was successfully carried out with fibroblast cell culture. It was observed that carbazole toxicity is less in comparison to anthracene fluorophore when the concentration of sample was increased. The carbazole amino acids were shown to be cytocompatible up to 50 μM concentration with the fibroblast cells and help in their proliferation. An increase in the carbazole amino acid concentration to 100 μM decreased the cell viability to 60%.
Human Dermal Fibroblasts cytocompatibility with carbazole amino acids (59b)

The biocompatibility for carbazole and anthracene containing fluorophores was tested with different concentrations, (Figure-21) and the result shows that carbazole is less toxic compared to anthracene fluorophore with the cancer cell lines tested. Anthracene showed more toxicity even in 25 µM concentration and when the concentration was increased to 250 µM, cell was completely destroyed. But carbazole fluorophore showed good biocompatibility at 25 µM and 50 µM and (Figure-22) moderate biocompatibility at 150 µM and 250 µM.
Fluorescence of carbazole and anthracene amino acids

Carbazole amino acid exhibited green fluorescence with the tested fibroblast and MCF7 cell lines. The anthracene amino acid showed complete cytotoxicity on the fibroblast cells. When the carbazole amino acid showed higher compatibility and lesser fluorescence, the anthracene amino acid showed better fluorescence and lower compatibility. At lower concentrations around 11 µM, anthracene amino acids showed blue fluorescence and good cancer cell cytocompatibility. With the carbazole amino acid the fibroblast cell lines retained their morphology and with all the amino acids the cancer cell lines showed good morphology with no effect of toxicity.

Carbazole amino acids (59b) incubated human fibroblasts

Thus the fluorescent amino acids can be used for fluorescently marking various cell lines and in particular carbazole amino acids are ideal for fluorescently marking the cells without much cytotoxicity in both cancer cells and in the normal fibroblast cell lines (Figure-23).
Carbazole amino acids (59b) incubated MCF7 breast cancer cell lines

Figure-24

Blue fluorescent anthracene amino acids (47c, 47d, 50a, 50c) incubated MCF7 cell lines

Figure-25

Cell line and culture

Human Breast cancer MCF-7 cell lines and Lung Cancer cell lines (A459) 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.

In vitro assay for cytotoxicity activity (MTT assay)

The cytotoxicity of samples on MCF-7 cells was determined by the MTT assay (Mosmann et al., 1983). Cells (1 × 10⁵/well) were plated in 5mL of medium/well in 6-well plates (Costar
Corning, Rochester, NY). After 48 h incubation, the cells reach the confluence. Then, cells were incubated in the presence samples for 24 – 48 h at 37°C. After removal of the sample solution and washing with phosphate-buffered saline (pH 7.4), 1mL/well (5mg/mL) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl–tetrazolium bromide cells (MTT) phosphate-buffered saline solution was added. After 4h incubation, 0.04M HCl/ isopropanol was added. Viable cells were determined by the absorbance at 570nm. Measurements were performed and the concentration required for a 50% inhibition of viability (IC\textsubscript{50}) was determined graphically. The absorbance at 570 nm was measured with a UV- Spectrophotometer using wells without sample containing cells as blanks. The effect of the samples on the proliferation of MCF-7 cancer cells was expressed as the % cell viability, using the following formula:

\[
\% \text{ cell viability} = \frac{A_{570} \text{ of treated cells}}{A_{570} \text{ of control cells}} \times 100 \%
\]

**Human Dermal Fibroblast (HDF) culture**

HDF were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 10% FBS and 1% antibiotic and antimycotic solutions (termed as normal growth media) in a 75 cm\textsuperscript{2} cell culture flask. Cells were incubated at 37 °C in a humidified atmosphere containing 5% CO\textsubscript{2} for 6 days and the culture medium was changed once in every 3 days. 11, 25, 50, 100 and 250 µM concentration of fluorescent amino acids were placed in 24-well plate and subsequently added DMEM overnight before cell seeding. HDF were grown to confluency and then the cells were detached by adding 1 ml of 0.25 % trypsin containing 0.1% EDTA. Detached cells were centrifuged, counted by trypan blue assay using a hemocytometer, and seeded in the fluorescent amino acid media at a density of 10,000 cells per well.
Cell proliferation assay

Cell proliferation on the substrates with fluorescent amino acids was determined using the colorimetric 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2(4-sulfophenyl)-2H tetrazolium (MTS) assay (CellTiter 96 AQueous One solution; Promega). The reduction of yellow tetrazolium salt [3-(4, 5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2(4-sulfophenyl)-2H tetrazolium] in MTS to form purple formazan crystals by the dehydrogenase enzymes secreted by mitochondria of metabolically active cells formed the basis of this assay. The formazan dye shows the absorbance at 490 nm and the amount of formazan crystals formed is directly proportional to the number of cells. After culturing the cells for a period of 3, 6 and 9 days, they were rinsed with PBS to remove unattached cells and incubated with 20% MTS reagent in serum-free medium for a period of 3 h at 37 °C. Absorbance of the obtained dye was measured at 490 nm using a spectrophotometric plate reader (FLUOstar Optima, BMG Lab Technologies, Offenburg, Germany).

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 all the 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 sps.

Among the Gram +ve no significant difference was observed between the Bacillus genera and growth inhibition was less with Staphylococcus aureus. Among all the organisms, maximum growth inhibition was observed with Micrococcus and Candida.
*albicans*. Following is the results on zone of inhibition (*Table-2*) measured in mm along with sample ID.