Chapter 2

Literature review

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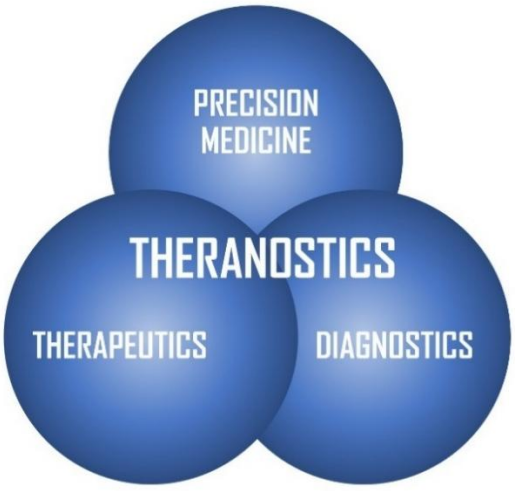
2.1 Theranostics fluorescent probes

The term “theranostic” was coined in 2002 by Funkhouser, which allows the ingenious integration of diagnostic imaging capability, combining the modalities of therapy, and has gained growing interest in both research and clinical areas (58, 59). The ultimate objective of theranostics is to combine diagnostic and therapeutic capabilities in a single agent. Moreover, it should gain the ability to image as well as monitor the diseased tissue, delivery kinetics, and drug efficacy (59). Several imaging techniques, such as optical (absorption, fluorescence, or bioluminescence), MRI, nuclear (PET, SPECT), photoacoustic, and ultrasound, are widely applied in theranostics (60). Aggregation-induced emission (AIE)-based theranostics provides the advantages of high emission efficiency in the aggregate state, promising photostability, large Stokes shift, low background noise, and on-site activation ability (61).



2.2 Fluorescent probes with classical push-pull architecture

Several fluorescent probes detecting Aβ species shared the common structural framework containing push-pull architecture with the electron-donor (D) group and electron-acceptor (A) group interconnected with the conjugated linker group (π bridge). The

fluorescent probes with near infrared region are mainly designed with the objective of reducing the gap between the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO), which is associated with absorption and emission wavelengths. The "donor-π-acceptor" based push-pull architecture strategy is commonly used in order to decrease the HOMO-LUMO gap. The existence of both the A and D groups, interconnected with the π-bridge, makes the system more polarized, and the ground state gets closer to the excited state; hence, the lower energy is required for the absorption and emission transitions, resulting in a bathochromic shift (26).

Table 2.1 Fluorescent properties & binding affinities of fluorescent probes with classical push-pull architecture.

Sr. No.	Name & Chemical structure of probes	$\lambda_{em1}/\lambda_{em2}$	Binding affinity for Aβ (Kd) (nM)	Φ (%)	References
1.	DANIR-22b	520/650	12.96 ± 3.19	0.3[a]/0.2[b]	(62)
2.	DCM-AN	725/661	0.85 μM	0.06	(63)
3.	Probe-20	709/648	1.95 μM	-	(64)
4.	Probe-4b	721/642	8.5 ± 2.5	19.8[a]/9.5[b]	(65)
5.	CRANAD-58	-/675	13.49 ± 0.84	9.6[a]	(66)
6.	Probe 9	668/697	14.57 ± 1.27	20.31[a]	(66)
7.	JP-1	-/670	0.264 uM	0.14	(67)
8.	QNO-AD-3	-/635	23.84 ± 1.703	0.10[b]	(68)
9.	DBAN-SLM	615/685	20.1 μM	0.02	(69)
10.	4PmNO-2	712/632	23.64 ± 1.08	61.4[b]	(70)
11.	CX1	-/675	230.8	1.6	(71)

λ_{em1}: emission maxima of probes, λ_{em2}: emission maxima upon interaction with Aβ, measured in [a]DCM, [b]PBS, and [c]Ethanol.

The optical and biological properties of probes are significantly affected by the alteration in the architecture of molecules, such as modification in electron-donor and acceptor

groups, an increase or decrease in the degree of conjugation of the π bridge (27). Different scaffold features are utilized to develop novel donor-π-acceptor-based fluorescent probes to improve selectivity, binding affinity, and essential biological and optical properties. In general, most of the probes with polyene chains show a rise in emission wavelengths with the increase in length of the π-conjugated system. However, the other structural features can also affect the same. The recently reported potential examples of the fluorescent AD probes have been systematically updated in the table (**Table 2.1, Figure 2.1**).

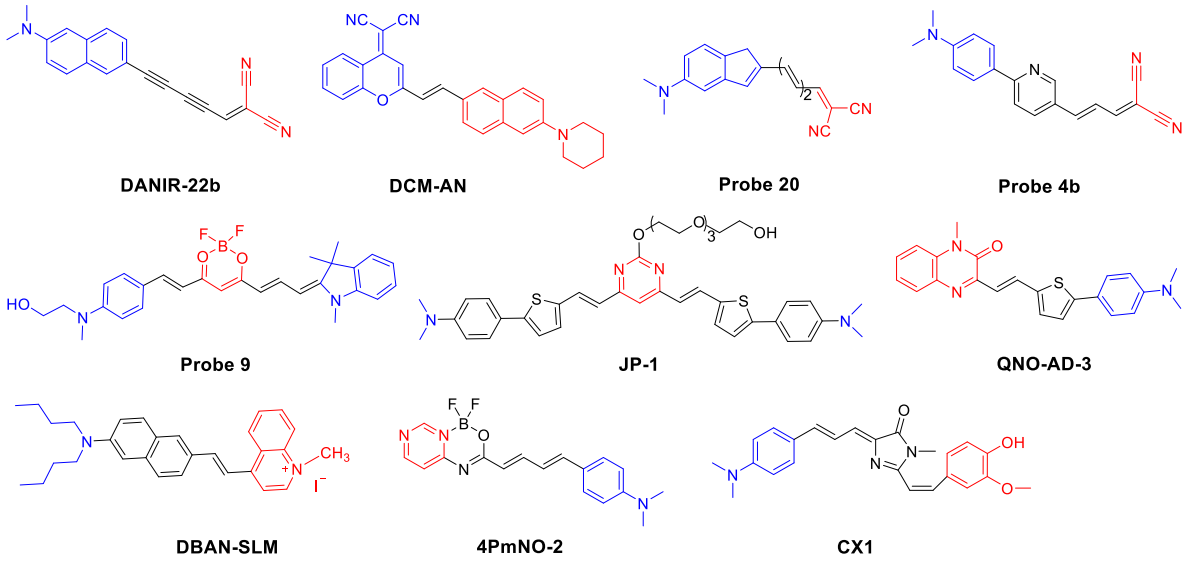


Figure 2.1. Chemical structures of recently reported classical push-pull architecture-based fluorescent probes derived from dicyanomethylene, curcumin, quinoxalinone, quinolone, and imidazole (Acceptor and donor groups were labelled in red and blue, respectively). Modifications at the donor, acceptors, and conjugated degree of the π system significantly affect probes' optical and biological properties.

2.3 Fluorescent probe designed with novel strategies

Although, numerous fluorescent probes for Aβ imaging were developed with the classical approach, the designed strategy mainly focused on the modifications in the architectures of electron donors and acceptors and the conjugated degree of the π bridge. However, to improve the practical usage and minimize some drawbacks of the classical approach, various novel design strategies were also utilized to develop fluorescent probes to detect

Aβ species (**Table 2.2, Figure 2.2**). To potentiate practical usage, different novel strategies have been employed, including AIE-based probes, theranostics probes for diagnosis and treatment, photoinduced electron transfer (PeT) and resonance energy transfer (RET) fluorescence regulation mechanisms-based probes, and twisted intramolecular charge transfer (TICT) devoted to designing multifunctional probes. The development of AIE-active probes for detecting Aβ species for bio-sensing and *in vivo* imaging has always been an interesting topic due to their bright fluorescence effect when aggregated, and weak fluorescence when separated in solution. The AIE effect is said to occur due to the restriction in the intramolecular rotation process and the presence of a non-planar configuration in its aggregate state, which undergoes π-π stacking interactions (72). The recently developed thiophene-derived AIE-based probe, DNTPH, is described in the section below. Theranostic probes are known to provide the benefits of both diagnostic and therapeutic activity simultaneously. Aβ is considered the therapeutic target and major neuropathological hallmark for AD and is therefore suitable for the theranostic approach. It shows the potential of monitoring pharmacokinetics and pharmacodynamics parameters, which pave the way to personalized medicine. The theranostic strategy can address the challenge of early diagnosis and disease monitoring for an efficient therapy of AD. Various theranostics agents have recently been reported, including **F-SLOH**, **Gd(DOTA)-cyanine**, **DBAN-SLM,** and **DTB**. Another novel concept of PeT-based probes has attracted significant interest due to its high signal-to-noise ratios, where it demonstrated the fluorescence "off−on" mode through the intramolecular transfer of electrons from the fluorophore unit to the recognition element, which caused fluorescence quenching (73). The structure–property relationship that governs this phenomenon is explained by two processes, acceptor-excited PeT (a-PeT) and donor-excited PeT (d-PeT), depending on the electron transfer direction. The transfer

of electrons occurs between the recognition group and the fluorophore in a-PeT and from the fluorophore to the recognition group for d-PeT (74). e.g. **Rho1-Cu−Rho4-Cu.** Recently emerged, RET-based probes exhibit non-radiative energy transfer between the donor group and the recipient in appropriate proximity, such as Förster resonance energy transfer (FRET) and chemiluminescence resonance (75). FRET is a process of non-radiative energy transfer through long-range dipole–dipole interactions between a pair of donor and acceptor due to their readily tuned structural features. It shows the inherent sensitivity to the environment and probe structure due to the presence of a distance-dependent energy transfer process (76).

Table 2.2 Fluorescent properties & binding affinities of fluorescent probes designed based on different strategies.

Sr. No.	Name & Chemical structure of probes	$\lambda_{em1}/\lambda_{em2}$	Binding affinity for Aβ (Kd) (nM)	Φ (%)	References
1.	F-SLOH	620/643	1.90 μM	0.6	(77)
2.	Gd (DOTA)-cyanine dyad	634−650/-	90-300 μM	0.0057	(78)
3.	MA-SLM	580/661	0.578	0.3±0.03	(79)
4.	DNTPH	708/710	146	7.6	(80)
5.	DTB	550-650/-	-	2.8%	(81)
6.	MSN-Lf@SZIs	593/-	507.6 ±94.7 5	19.2	(82)
7.	Rho1-Cu−Rho4-Cu	540/594	23.4	1.2	(83)
8.	AD-1	704/-	356.1 ± 112.7	0.38	(84)
9.	CAQ	693/-	78.89	0.011	(85)
10.	CRANAD-2	685/-	38.69 ± 2.77	0.006	(86)
11.	Probe 8b	667/-	91.2 ± 3.28	-	(87)
12.	Probe-4	652/613	2.83 μM	-	(88)

λ_{em1}: emission maxima of probes, λ_{em2}: emission maxima upon interaction with Aβ, measured in [a]DCM, [b]PBS, and [c]Ethanol.

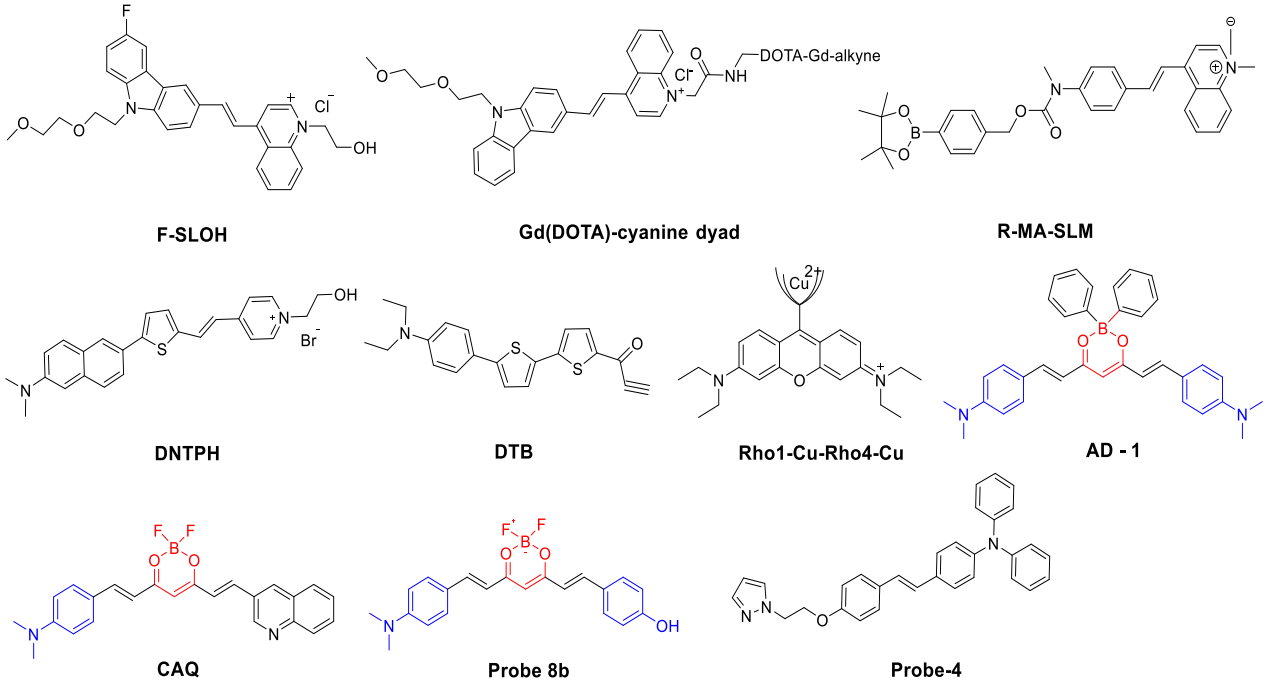


Figure 2.2. Chemical structures of recently reported fluorescent probes, designed on the basis of different strategies and mainly derived from cyanine, thiophene, thioflavin-T, rhodamine, and curcumin scaffolds (Acceptor and donor groups were labelled in red and blue, respectively).

2.4 Multi-target directed ligands (MTDLs)

Designing multi-target directed ligands (MTDLs) has been successful in complex diseases, viz., cancer, HIV, and hypertension, and may also be fruitful for neurodegenerative disorders (89). An innovative strategy to design single chemical entities able to simultaneously modulate more than one target is called "multitarget-directed ligands (MTDLs)" (90). MTDLs could be a valuable tool for targeting two or more disease progression mechanisms in AD to improve therapeutic efficacy and (or) safety. Two key steps are involved in developing MTDLs, including identifying a group of targets that can be modulated together and offer enhancement in the therapeutic effect compared to the single target. In the second step, a single chemical structure is designed in such a way that it is capable of modulating or interacting with all selected targets (91). Different strategies can be applied for the actual chemical design, such as selecting the

molecules active at one of the selected targets, engineering them for other targets, and identifying completely novel scaffolds capable of binding all the designated targets (91, 92). The important approach, scaffold hopping, is considered an extension of traditional bio-isosteric replacement strategies. It includes the redesign of the core structural moiety with a number of different structural changes ranging from heterocyclic substitution to major structural overhauls (93). The scaffold hopping strategies are widely applied in central nervous system drug discovery (93).

2.4.1 Rational Combination of Multiple Targets for MTDLs

The target combination for the discovery of the MTDLs is very crucial; the ideal combination of the target may provide superior therapeutic efficacy through a synergistic effect. Several approaches were applied for the rational combination of multiple targets. In general, combinations are based on clinical observations, phenotypic screening, and *in silico* techniques (94). In clinical trials, drug cocktail topics are most commonly referred to in combination therapies, but some limitation of complex PK profiles and drug−drug interaction limits their clinical applications. However, clinical observation may suggest the target combination (validated by drug cocktails) that enhances efficacy (95). Phenotypic screening is another important approach for target combination, where a large number of compound combinations can be used for screening on cellular, tissue, and animal models for synergies (96). However, tremendous animal experiments are required for high throughput of putative compounds or target combinations. Lastly, *in-silico* technique is also a viable approach for screening a suitable target combination. Several *in silico* methodologies are available, such as analysis of biological target networks via machine learning and network pharmacology methods (97). Wet-lab validation is further required to confirm its biological foundation and feasibility.

2.4.2 Rational Design of MTDLs

In general, four strategies are commonly used for the design of the MTDLs, including merged-pharmacophore mode, fused-pharmacophore mode, noncleavable linked-pharmacophore mode, and cleavable linked-pharmacophore mode (98). The merged-pharmacophore mode can be utilised to design a dual-target inhibitor, if the binding sites of the two targets can bind ligands with similar geometry and pharmacophore distribution (99). Its implementation is difficult if the two compounds do not have similar structures or if they have dissimilar binding regions of target proteins. Secondly, the pharmacophores of the two lead compounds can be fused directly or connected with a linker in order to maintain the original pharmacophore binding, where, two key pharmacophores can be fused directly, if the binding regions are large or adjacent to the solution (100). Another linked-pharmacophore strategy can be used if the binding regions are small and deep inside the protein (101).

2.4.3 MTDLs lead optimization

The structural optimization of the identified lead compound is challenging, where the major objective is to generate high potency on two molecular targets and demonstrate a balanced activity profile on these targets of interest. In the phase of lead optimization, mainly two approaches are utilized, including the design-in and design-out approach (102). In the Design-in approach, the pharmacophore of one compound is designed into the other according to molecular architecture and functional groups that are crucial for interaction with the biological targets of interest. Preferably, the two selective lead compounds share sufficient similarity (94). The design-out strategy for the development of multiple ligands with a designed target modulation profile follows the completely opposite direction from the design-in approach.

The strategy starts from an unselective ligand that modulates multiple biological targets, including the targets of interest, rather than using selective compounds and merging their pharmacophores by stepwise structural adaptation. The design-out approach mainly generates the ligands by enhancing their selectivity towards the desired target of interest by reducing the potency on the undesired targets through directed structural changes (102).

2.5 Computer-aided drug discovery

The approaches and methodologies used in the design of drugs have undergone transformation over a period of time. At present, drug design approaches accomplish and drive new technological advances to solve the varied, new, difficult setbacks that occur along the path to drug discovery. As advances in structural genomics, bioinformatics, and computational power continue to expand, further successes in structure-based and ligand-based drug design are likely to follow. Comparatively, a lower amount of time, cost, and labour is spent in the computational drug design, and can have a huge impact on the discovery of new drug molecules. In the drug discovery process, the identification of molecules that show significant interactions with the therapeutic targets is of paramount importance (103, 104). Structure-based drug design (SBDD) is a highly validated and essential design approach in the modern drug discovery process (105, 106). It directs the discovery of a lead compound that could show potent target inhibitory activity through a faster and cost-efficient approach. In order to rapidly achieve new drug compounds for clinical use, a combination of SBDD, virtual drug screening, and high-throughput screening approaches could be employed for drug discovery. In the discovery and optimization of the lead, SBDD is a more specific, efficient, and rapid process. The knowledge of 3D structures of the biological targets is a prerequisite for SBDD. Due to the advances in bioinformatics and the completion of the Human Genome Project, a vast

number of 3D structures of the target proteins are now available. SBDD methods analyses the information of 3-dimensional macromolecular targets, mostly of proteins or RNA, to identify key sites and interactions that are essential for their respective biological function. It is mainly used for binding energy analysis, interaction of ligand-protein, and evaluation of the conformational changes during the process of docking (107, 108, 109, 110, 111). If the information of the therapeutic target structure is not available, the alternative approach used is called ligand-based drug design (LBDD). LBDD is applied on the principle that structurally similar molecules are likely to have similar properties, where structural information and bioactivity data for small molecules are sufficient (112).