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DESIGN, SYNTHESIS AND PHOTOPHYSICAL EVALUATION OF CARBAZOLE-BASED HETEROCYCLIC FLUOROPHORES FOR BIOMEDICAL APPLICATIONS

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ABSTRACT

Carbazole-based heterocyclic fluorophores offer a versatile route to fluorescent probes because the electron-rich carbazole framework can be combined with acceptors and substituents that modify optical response and biological distribution. This paper examines primary literature published and develops a matched molecular design for subsequent experimental evaluation. Published measurements for the mono-cyanine SPBN and bis-cyanine VPBN are used to compare solvent-dependent fluorescence, peak molecular brightness and two-photon action cross-sections. Their fluorescence quantum yields are lower in phosphate buffer than in dimethyl sulfoxide, while their relative performance depends on whether one-photon brightness or two-photon excitation is considered. These observations are interpreted alongside earlier studies of carbazole–BODIPY, carbazole–coumarin, carbazole–benzothiazole and styryl systems. A three-member carbazole–vinyl series is proposed to examine acceptor extension and side-chain polarity through controlled structural changes. The evaluation framework connects chemical identity and purity with calibrated spectroscopy, aggregation assessment, cellular localisation and tolerance. The analysis identifies environmental response as a central design variable and shows why molecular absorption alone cannot establish the usefulness of a biomedical fluorophore.

Keywords: Carbazole; heterocyclic fluorophores; donor–acceptor systems; fluorescence quantum yield; two-photon excitation; bioimaging.

INTRODUCTION

Fluorescence provides a means of examining molecular distribution and local chemical environments within biological specimens. Its usefulness depends on more than the position of an emission maximum. Excitation efficiency, fluorescence yield, photostability and the amount of probe reaching the intended compartment jointly determine the measured signal. The design problem is therefore to connect an optical response with a chemically defined structure and a reproducible biological setting.

Carbazole supplies an electron-rich aromatic unit whose substitution can be varied at nitrogen and at the peripheral carbon positions. Yang et al. demonstrated organelle imaging with carbazole-based cyanines [1], while Liu et al. investigated cationic carbazole donor–acceptor compounds for mitochondrial imaging [2]. These studies establish useful precedents for combining conjugated acceptors with substituents that influence cellular distribution. They do not imply that every positively charged carbazole derivative will share the same intracellular destination.

Other heterocyclic combinations broaden the available design space. Maeda, Todaka and Ema developed carbazole-based BODIPYs with tunable optical properties [3]. Zheng et al. introduced a fused carbazole–coumarin platform for two-photon sensing and reported a carbon-monoxide-responsive derivative [4]. Yu, Li and Fei examined the optical behaviour

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and protein labelling of carbazole–benzothiazoles [5], and Sekar et al. linked solvent-dependent fluorescence in carbazole styryl dyes with density functional calculations [6]. Together, these studies show how the attached heterocycle and the surrounding medium can alter the behaviour of a common donor framework.

The present analysis addresses two related questions. First, which optical descriptors provide an informative comparison when published measurements are obtained under defined conditions? Second, how can those observations guide a small synthetic series in which the effects of acceptor identity and side-chain polarity remain interpretable? The contribution is a comparative literature analysis and a prospective experimental design; the proposed compounds are not assigned measured properties.

LITERATURE SCOPE AND ANALYTICAL APPROACH

The analysis is restricted to primary studies published on or before 31 December 2015. Sources were selected for their relevance to carbazole-containing heterocycles, molecular synthesis, photophysical characterisation or biological imaging. This focused selection is intended to establish chemical and methodological precedents rather than to estimate the prevalence of particular properties across the entire field.

The quantitative comparison uses SPBN and VPBN from the same published study [1]. Four compound–medium combinations are retained: each dye in pH 7 phosphate buffer and in dimethyl sulfoxide (DMSO). Peak molecular brightness and wavenumber Stokes shifts are calculated from the displayed absorption coefficients, quantum yields and spectral maxima. Maximum two-photon absorption cross-sections are considered separately from their fluorescence-weighted products. This separation avoids treating one-photon and two-photon performance as interchangeable quantities.

3 MOLECULAR DESIGN AND SYNTHETIC STRATEGY

A matched series provides a direct way to investigate selected structural variables. The proposed compounds P1–P3 retain a carbazole donor attached through a vinyl unit to a cationic nitrogen heterocycle. P1 and P2 compare pyridinium and quinolinium acceptors while retaining an N9-ethyl substituent. P1 and P3 compare an ethyl group with an ether-containing side chain while retaining the pyridinium acceptor. Bromide is specified as the common counterion.

Table 1. Proposed carbazole–vinyl fluorophore series.

Compound	Carbazole N9 group	Cationic acceptor
P1	Ethyl	N-Methylpyridinium; vinyl linkage at C4
P2	Ethyl	N-Methylquinolinium; vinyl linkage at C4
P3	2-(2-Methoxyethoxy)ethyl	N-Methylpyridinium; vinyl linkage at C4

The prospective synthesis begins with N9 substitution of carbazole and preparation of the corresponding carbazole-3-carbaldehyde. Quaternisation of a methyl-substituted pyridine or quinoline would supply the activated methyl group required for condensation with the aldehyde. Formation of the vinyl linkage would connect donor and acceptor. The cyanine chemistry in [1] provides an earlier precedent for the condensation of carbazolyl aldehydes with functionalised methylpyridinium salts. The conditions and isolation procedures for P1–P3 would nevertheless require individual optimisation.

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Reaction monitoring would combine chromatography with mass spectrometry so that conversion is distinguished from isolated recovery. Residual aldehyde, unreacted heterocycle, inorganic salts and geometric isomers may complicate a strongly coloured reaction mixture. Purification would therefore be assessed by the composition of the recovered material rather than by colour or a single thin-layer chromatographic spot. Proton and carbon NMR, supported where necessary by two-dimensional experiments, would establish the intended connectivity. Alkene coupling constants would assist assignment of the conjugated linkage.

The BODIPY work of Maeda et al. offers a complementary synthetic precedent involving sequential iridium-catalysed borylation, Suzuki–Miyaura coupling and boron complexation [3]. It illustrates how a carbazole unit can be incorporated into a different chromophore architecture. The resulting optical properties cannot be transferred quantitatively to a vinylpyridinium compound, because the electronic framework and conformational constraints differ.

For the matched series, acceptor extension is expected to alter the energies and transition strengths of the charge-transfer states, whereas the ether side chain may principally affect solvation and distribution. These are working hypotheses. Either change could also influence conformation or aggregation, and improved handling in aqueous medium could accompany reduced cellular uptake. Comparing the same counterion, comparable purity and matched concentration would help separate the intended structural effects from changes in sample preparation.

Computational screening would examine accessible conformations before selecting a single optimised geometry. Ground-state and time-dependent density functional calculations would be compared with a related compound whose absorption is known. Sekar et al. provide a precedent for combining measured solvent response with calculated electronic properties in carbazole styryl dyes [6]. In the proposed study, differences between calculated and measured transitions would inform model choice; calculated orbital gaps would not be substituted for experimentally determined absorption energies.

PHOTOPHYSICAL EVALUATION

Absorption measurements would first establish a concentration interval over which the Beer–Lambert relationship is maintained. Fluorescence spectra would then be recorded with matched temperature and solvent conditions, using correction for the wavelength response of the detection system. Dilution experiments would identify concentration-dependent spectral changes and reduce the risk of inner-filter effects or self-absorption. Quantum yields would be measured against a suitable reference or with a calibrated integrating sphere, following the methodological principles described by Brouwer [7] and Würth et al. [8].

For relative quantum-yield measurements, integrated emission must be related to the fraction of excitation light absorbed, with the appropriate refractive-index correction. A brighter spectrum at the same nominal concentration can result from stronger absorption rather than a larger fluorescence quantum yield. Independent solution preparations, calibration checks and documented excitation conditions are therefore necessary for a useful comparison. Reference and sample measurements would be obtained within the linear operating range of the instrument.

Peak one-photon molecular brightness and wavenumber Stokes shift are defined here as:

$$B_{max} = \varepsilon_{max} \times \Phi_F \quad (1)$$

$$\Delta\tilde{\nu} = 10^7 \times (1/\lambda_{abs} - 1/\lambda_{em}) \quad (2)$$

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In these expressions, ε_{max} is the molar absorption coefficient in M^{-1} cm^{-1}, Φ_F is dimensionless and the wavelengths are in nm; $\Delta\tilde{\nu}$ is obtained in cm^{-1}. A microscope-specific estimate would use the absorption coefficient at the actual excitation wavelength. Peak brightness is consequently a molecular screening descriptor rather than a complete prediction of recorded cellular intensity.

For two-photon excitation, the fluorescence-weighted action cross-section is $\sigma_2\Phi_F$, where σ_2 is the two-photon absorption cross-section. A large σ_2 can be partly offset by a small fluorescence yield. Comparisons at individual absorption maxima are useful for examining molecular capability, but a practical imaging comparison would additionally use a common excitation wavelength, controlled laser power and equivalent collection conditions.

Time-resolved measurements would establish whether changes in steady-state yield are accompanied by changes in fluorescence lifetime. For a single population with monoexponential fluorescence decay, the radiative and nonradiative rate constants can be estimated from $k_r = \Phi_F/\tau$ and $k_{nr} = (1 - \Phi_F)/\tau$. A multiexponential decay would instead require examination of its components and residuals. The adequacy of the kinetic model should be established before assigning a structural change to a particular deactivation pathway.

Environmental screening would vary solvent composition, concentration, temperature and viscosity in a controlled sequence. Measurements in buffered medium would be accompanied by inspection of absorption line shape and, where particles are present, light-scattering measurements. Protein-containing media would be examined separately because binding can change the effective environment of a dye. Photostability would be evaluated under the intended excitation conditions, with absorbed-light exposure recorded so that unequal excitation does not distort the comparison.

PUBLISHED MEASUREMENTS AND COMPARATIVE ANALYSIS

Table 2 reproduces the selected measurements for SPBN and VPBN reported by Yang et al. [1]. The paired media allow spectral position and fluorescence efficiency to be examined within each compound. Figure 1 displays the spectral maxima and quantum yields without implying a continuous spectral profile.

Table 2. Published photophysical measurements for SPBN and VPBN [1].

Dye	Medium	λ_{abs}(nm)	ε_{max}(10^4 M^{-1} cm^{-1})	λ_{em}(nm)	Φ_F
SPBN	Buffer, pH 7	424	1.99	600	0.020
VPBN	Buffer, pH 7	443	4.26	582	0.003
SPBN	DMSO	435	1.80	603	0.370
VPBN	DMSO	453	3.92	618	0.030

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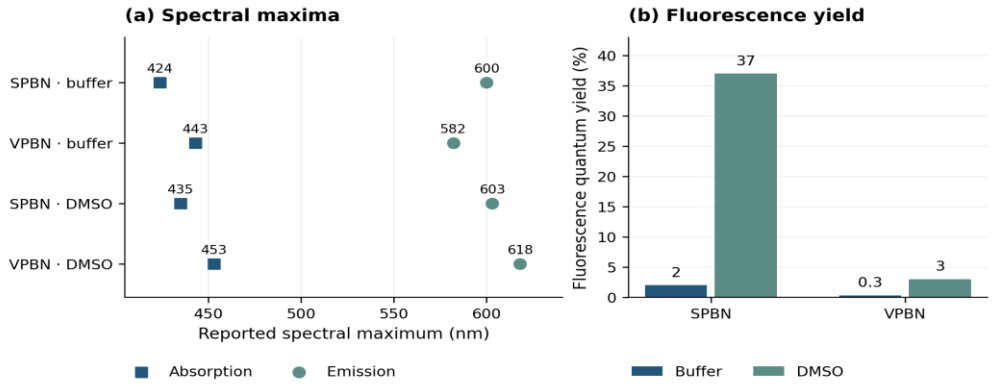


Figure 1. Spectral maxima and fluorescence quantum yields of SPBN and VPBN in phosphate buffer (pH 7) and DMSO, replotted from [1]. Markers in panel (a) represent peak positions rather than spectral profiles. Quantum yields in panel (b) are expressed as percentages.

Calculations from Table 2 give peak brightness values of 398 and 127.8 M^{-1} cm^{-1} for SPBN and VPBN in buffer, compared with 6660 and 1176 M^{-1} cm^{-1} in DMSO. The corresponding calculated Stokes shifts are approximately 6918 and 5391 cm^{-1} in buffer, and 6405 and 5894 cm^{-1} in DMSO. These derived quantities show that absorption strength, spectral separation and fluorescence efficiency describe different aspects of performance.

Figure 2 relates one-photon brightness to the two-photon descriptors. From the reported maximum σ_2 values of 243 and 5130 GM, the calculated buffer-phase action cross-sections are 4.86 and 15.39 GM for SPBN and VPBN, respectively. Thus, the ranking by peak one-photon brightness differs from that by maximum two-photon action cross-section. The latter values refer to different excitation maxima, 890 and 910 nm, and do not establish an equal-wavelength imaging comparison [1].

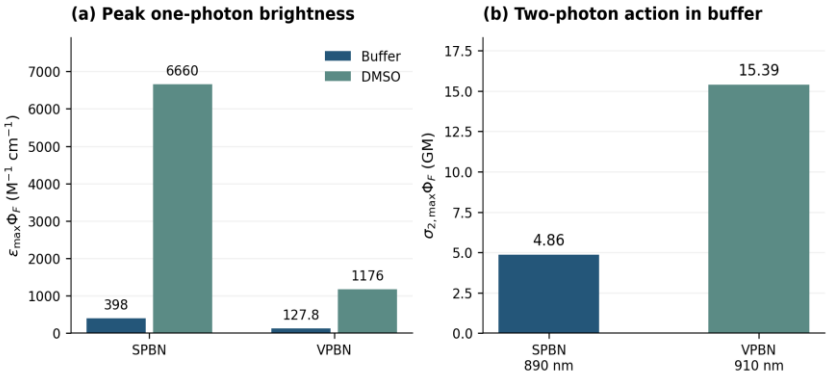


Figure 2. Calculated peak molecular brightness (a) and maximum two-photon action cross-section in phosphate buffer (b), using the measurements in [1]. The products σ_2,maxΦF use reported σ_2,max values of 243 GM for SPBN at 890 nm and 5130 GM for VPBN at 910 nm. The excitation maxima differ; this is not a common-wavelength comparison. One GM equals 10^{-50} cm^4 s $photon^{-1}$ $molecule^{-1}$.

This difference has a direct design implication. Increasing the interaction of a molecule with incident light is useful only to the extent that the absorbed energy produces the desired signal. For a practical imaging experiment, the molecular response must also overlap with the available laser and detector range. Optimisation should therefore begin with the intended excitation mode and specimen rather than with a universal ranking of dye brightness.

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The solvent comparison also motivates closer examination of conformational and intermolecular effects. A change in medium can alter the stabilisation of an excited state, molecular motion, association and specific binding. The displayed data establish environmental dependence, but do not isolate one mechanism. For P1–P3, concentration and temperature series would help determine whether an apparent solvent effect persists when aggregation and thermal relaxation are controlled.

Earlier work on carbazole–benzothiazoles provides a related biological perspective. Yu et al. investigated BEC and DBEC, including solvent-dependent spectra, stability and interaction with bovine serum albumin [5]. Protein-associated fluorescence enhancement suggests that a dye may behave differently in a macromolecular environment from its behaviour in bulk solvent. Such an observation supports binding experiments, but does not by itself identify an intracellular target or establish selective protein recognition.

The carbazole–coumarin platform examined by Zheng et al. represents a different objective: a molecular response intended to report an analyte rather than merely label a location [4]. Its use in a reported carbon-monoxide sensing system illustrates how an optical scaffold can be coupled to a chemical recognition process. Evaluation of a related sensor would need to distinguish response to the analyte from response to its delivery reagent, reaction products and changes in the surrounding medium.

The broader comparison favours application-specific development. A localisation label benefits from a stable and reproducible signal across the environments encountered during imaging. An environmental sensor requires a response that is both calibrated and distinguishable from competing influences. The same structural flexibility that increases sensitivity in one application may reduce interpretability in another.

BIOMEDICAL EVALUATION OF THE PROPOSED SERIES

Cellular evaluation would begin with a concentration and exposure series spanning detectable fluorescence and acceptable cellular tolerance. A pilot interval of 0.1–10 μM could be examined, subject to solubility and initial viability findings. Unstained, vehicle-treated and single-channel controls would accompany the experiments. Uptake time, washing, serum content and excitation dose would remain consistent across P1–P3 so that differences are not introduced by the imaging procedure.

Cationic donor–acceptor compounds reported by Liu et al. provide an earlier basis for considering mitochondrial accumulation [2]. For the proposed series, localisation would be tested by comparison with an established organelle marker. Background correction and spectral bleed-through controls would precede quantitative colocalisation analysis. Comeau, Costantino and Wiseman demonstrated the dependence of colocalisation accuracy on image characteristics and analysis method [9]; a merged colour image alone would therefore be insufficient to establish targeting.

Cellular signal would be interpreted together with uptake and retention. Greater fluorescence could reflect more intracellular compound, an increase in quantum yield after binding, or accumulation within a smaller volume. Washout measurements and, where feasible, independent chemical quantification would distinguish these possibilities. The ether-containing derivative is particularly useful for this purpose because improved aqueous handling need not correspond to increased intracellular accumulation.

Tolerance would be assessed under both dark and illuminated conditions, with controls for direct interference between the fluorophore and the assay readout. Viability measurements would be considered alongside morphology and recovery following exposure. Independent

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cultures would define the biological replication unit, while fields collected from the same culture would be treated as nested observations. This design would connect fluorescence performance with the conditions under which the specimen remains suitable for interpretation.

CONCLUSION

The literature available establishes several chemically distinct approaches to carbazole-based biomedical fluorescence. The quantitative comparison shows that solvent, fluorescence yield and excitation mode can change the ranking of closely related probes. These observations favour a development strategy in which acceptor identity and side-chain polarity are varied within a matched series, followed by chemical verification and calibrated measurements in the intended medium. The proposed P1–P3 study would determine whether those structural changes improve useful cellular contrast and whether any gain results from intrinsic photophysics, uptake or localisation. Its central objective is a reproducible relationship between molecular structure and imaging behaviour.

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