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Development of indazolylpyrimidine derivatives as high-affine EphB4 receptor ligands and potential PET radiotracers

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ABSTRACT

Due to their essential role in the pathogenesis of cancer, members of the Eph (erythropoietin-producing hepatoma cell line-A2) receptor tyrosine kinase family represent promising candidates for molecular imaging. Thus, the development and preparation of novel radiotracers for the noninvasive imaging of the EphB4 receptor via positron emission tomography (PET) is described. First in silico investigations with the indazolylpyrimidine lead compound which is known to be highly affine to EphB4 were executed to identify favorable labeling positions for an introduction of fluorine-18 to retain the affinity. Based on this, reference compounds as well as precursors were developed and labeled with carbon-11 and fluorine-18, respectively. For this purpose, a protecting group strategy essentially had to be generated to prevent unwanted methylation and to enable the introduction of fluorine-18. Further, a convenient radiolabeling strategy using [^{11}C]methyl iodide was established which afforded the isotopically labeled radiotracer in 30–35% RCY (d.c.) which is identical with the original inhibitor molecule. A spiro ammonium precursor was prepared for radiolabeling with fluorine-18. Unfortunately, the labeling did not lead to the desired ^{18}F-radiotracer under the chosen conditions.

1. Introduction

Members of the Eph receptor tyrosine kinase family play an essential role in the pathogenesis of cancer[1] and, therefore, they are promising candidates for noninvasive molecular imaging purposes, for example, by positron emission tomography (PET). These receptors belong to the largest family of receptor tyrosine kinases and they are divided into two groups, class A and B, depending on their structure and binding to the GPI-anchored class A ephrins or transmembrane proteins (ephrins) of class B.[2,3] Cellular survival, re-organization of the cytoskeleton, cell attachment, and motility was regulated due to the cellular communication system provided by Eph receptors and ephrins.[4] They are involved in physiological processes like embryonic development and angiogenesis[5,6] as well as in pathophysiological processes like tumorigenesis and tumorangiogenesis.[7]

EphB4 with its preferential ligand ephrinB2 is one of the most important Eph receptors. Pro-tumorigenic effects of EphB4 activation are discussed for prostate[8] and breast cancer, where EphB4 knockdown resulted in reduced tumor growth in mice.[9] Inconsistent outcome of EphB4/ephrinB2 signaling is also described for tumorigenesis,[10] with anti-tumorigenic effects of activated EphB4 in mouse melanoma and breast cancer cells, and reduction or loss of EphB4 expression in human colorectal tumors and invasive breast carcinoma.[11–13]

Several potent Eph kinase inhibitors were reported either based on high molecular weight compounds like peptides,[14–16] which block the extracellular domain of the appropriate receptor, or on small organic molecules,[17] which bind to the intramolecular ATP binding pocket. To date, only peptide-based radiotracers containing Cu-64 or In-111 like **I**[18,19] but also with F-18 like **II**,[20,21] which are specific for Eph receptors, are known for multimodal imaging purposes using PET or SPECT. Due to the favorable chemical and biological properties, small organic inhibitors (e.g., **IIIa** or **IV**) published by Bardelle et al. are the basis of our research (Scheme 1).[22–25] Recently, the novel fluorine-18-containing radiotracer **IIIb** based on the benzodioxolylpyrimidine structural motif was developed

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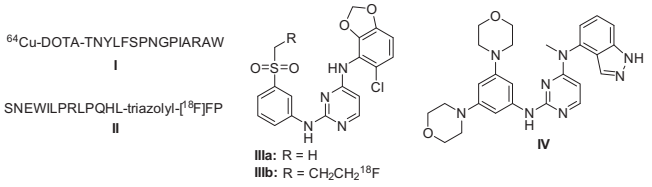
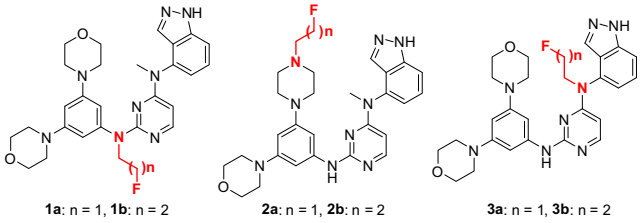


Scheme 1. Structures of selected EphB4 radiotracers and lead structure used in this paper.



Scheme 2. Proposed structures of fluorine-18 radiotracers and highlighted labeling positions.

and analyzed via in vitro and in vivo studies.[26] Unfortunately, despite the high affinity of the original inhibitor **IIIa** to the EphB4 receptor (IC_{50} = 90 nM, recombinant EphB4), the tracer **IIIb** showed a substantial uptake in tumor cells (A375) but no uptake in vivo in the respective tumor (A375) bearing mice. This can partially be explained due to its unfavorable (bio)chemical properties like a high lipophilicity ($c\log P$ = 3.77) or a rather low affinity. To overcome these problems, a novel lead structure was chosen based on a indazolylpyrimidine core containing inhibitor **IV** with a much higher affinity to the EphB4 receptor (IC_{50} = 1.3 nM, $c\log P$ = 3.42).[24]

In this regard, a convenient radiolabeling route to novel carbon-11 and fluorine-18 radiotracers including a synthesis plan for the insertion of protecting groups is pointed out. Prior to synthesis and labeling, in silico docking studies are accomplished for finding the best labeling position in case of the fluorine-18 introduction. Afterwards, the corresponding precursors and reference compounds for $^{11}C/^{18}F$ labeling are synthesized.

2. Results and discussion

2.1. In silico studies

Contrary to a radiolabeling with carbon-11 it is not possible to apply an isosterical labeling procedure with fluorine-18 due to the absence of fluorine-19 in the inhibitor lead molecule **IV**. To overcome this fact, single hydrogen atoms or functional groups like hydroxyl residues were normally replaced by fluorine-18/-19.[27] In most of the cases, this replacement leads to a change of the chemical and biological properties of the respective molecule. To fix this problem, acceptable labeling positions are necessary to determine using in silico docking experiments.

In general, three labeling positions were proven in the original molecule for the introduction of fluorine-18/19 as pointed out in Scheme 2. The simplest comprises the introduction of a fluoroalkyl moiety on the free secondary nitrogen atom of original inhibitor **IV** which would lead to structures **1a**,**b**. The second variant comprises a change of one of the morpholino groups of **IV** by a piperazine residue in the first step. In the second step, a fluoroalkyl chain was introduced into the piperazine moiety which gives **2a**,**b**. The last possibility comprehends change of the methyl group attached to the secondary nitrogen atom of **IV** by a fluoroalkyl group (**3a**,**b**). It was pointed out earlier, that both nitrogen atoms of the indazole moiety form hydrogen donor and acceptor bonds to the side chain carboxylate of the conserved αC-helix glutamate (Glu664) and the backbone NH of the DFG triad Asp758, respectively.[24]

First, we docked compound **IV** in available crystal structure that contains a similar inhibitor molecule. The crystal structure of the complex between EphB4 and the inhibitor N^4-(1*H*-indazol-4-yl)-N^2-(3-(methylsulfonyl)phenyl)pyrimidin-2,4-diamine (IMPPD) (pdb: 2x9f) was selected; it contains two conformations of the crystalized ligand. The structure shown in Figure 1 confirms the predicted binding mode of **IV** in the ATP binding site, with the pyrimidine N-1 and C-2 anilino N–H forming hydrogen acceptor and donor bonds to the hinge region at Met696 and the indazole heterocycle buried in the binding pocket, resulting in a

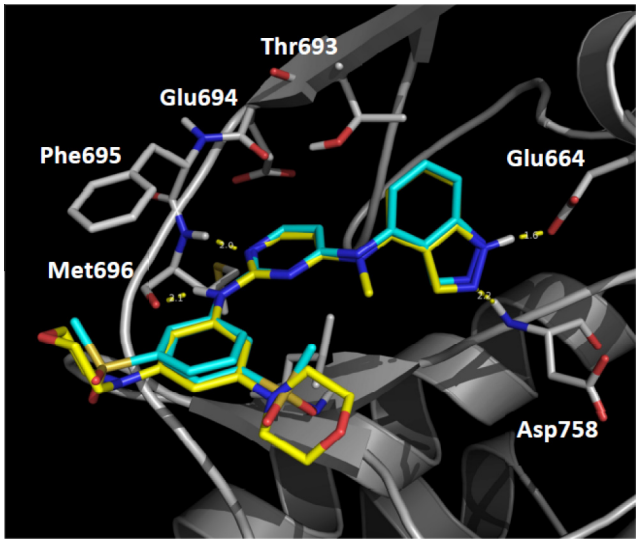


Figure 1. Conformational comparison of the ligand IMPPD (two conformations) from the crystal structure 2x9f (cyan) and compound **IV** (yellow).

'S-shaped' conformation. In this case, the indazole N01 NH and N02 nitrogen form hydrogen donor and acceptor bonds to the side chain carbonyl of Glu664 and the backbone NH of Asp758, respectively (Fig. 1). RMSD values considering the common heavy atoms between both conformations of IMPPD and docked conformation obtained for **IV** were 0.483 and 0.434 Å. The low RMSD values demonstrate that docking of **IV** reproduces IMPPD orientation.

On the basis of an X-ray structure analysis of the EphB4 receptor including a similar inhibitor molecule (IMPPD), the secondary nitrogen atom (highlighted in red in **1a**,**b**) of the original inhibitor **IV** has an important H-bond interaction with backbone of Met696 of the EphB4 receptor hinge region. According to this, it is expected that compounds **1a**,**b** should not have high interactions to the active site of EphB4 when ethyl or propyl groups are placed on this amine group. Therefore, compounds **1a**,**b** are not supposed to function as good EphB4 inhibitors.

Compounds **3a** and **3b** seemed to be favorable candidates. They have the same orientation with respect to the ligand from the reported X-ray structure, the change of methyl by F-propyl group should be tolerated since the pocket containing this group is large and contains hydrophobic residues, but the occupancy of this pocket by bigger hydrophobic groups can be unfavorable due to the presence of some hydrophilic groups. The fluoropropyl group of **3b** is located close to side chains of Leu747, Lys647 (catalytic lysine) and Val629, establishing hydrophobic interactions (Fig. 2).

Additionally, according to docking, **2a**,**b** seemed to be good candidates, too. They have also the same orientation with respect to the ligand from the reported X-ray structure and both the F-ethyl and the F-propyl group are tolerated. The F-propyl group of **2b** is oriented towards the solvent. It is difficult to explain the potency

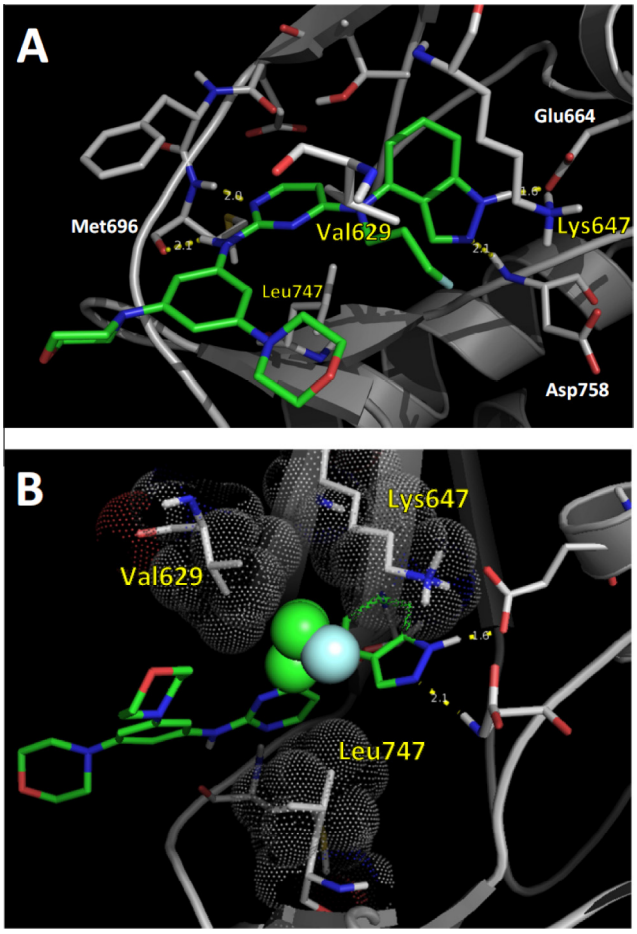


Figure 2. EphB4/compound **3b** complex. (a) Conformation proposed by docking; (b) interactions of the F-propyl group of the ligand (green spheres) with the hydrophobic wall composed by Val629, Lys647, and Leu747 (gray dots).

when groups are oriented to the solvent using modeling, because there are no interactions with protein residues. However, it is possible that **2a** and **2b** are potent inhibitors because the main interactions with the kinase (in the hinge region) are established for these compounds. Docking energy scores were similar for compounds **IV**, **2a**, **2b**, **3a**, and **3b** (Table 1).

Table 1
Docking energy scores for tested compounds

Compound	ICM scoring (kJ/mol)
IV	−435.65
1a	−373.51
1b	−396.93
2a	−454.92
2b	−429.99
3a	−430.03
3b	−440.55

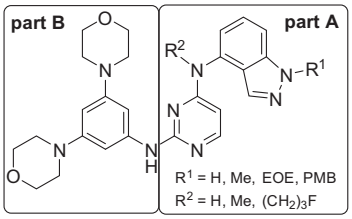


Figure 3. General composition of radiotracers, references and precursors.

2.2. Preparation

The synthesis path for all reference compounds and precursors for an isotopically labeled tracer with carbon-11 and a novel fluorine-18 containing radiotracer based on previous in silico studies was developed. In general, the inhibitor molecule **IV** as lead structure, the new radiotracers, the appropriate references and their corresponding precursors consist of two substructures A and B (Fig. 3) which were prepared separately.

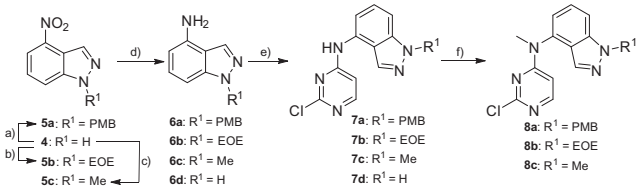
The first substructure (part A) of the compounds was prepared starting from indazole derivative **4**.[28] The development of a protecting group strategy was essential to avoid unwanted alkylation reactions at the nitrogens of indazole **4** in case of the introduction of carbon-11, which will be accomplished using [^{11}C]CH_3I, and further for the preparation of fluorine-18 precursors and references (see later). Thus, the *p*-methoxybenzyl (PMB) as well as the ethoxyethyl (EOE) group were chosen. Compound **5a** (1*H*-isomer) was yielded with 48% when **4** was reacted with *p*-methoxybenzyl chloride whereas compound **5b** was obtained in 80% yield when **4** was treated with ethyl vinyl ether under acidic conditions after a modified procedure published by Slade et al.[29] Additionally, methylated compound **5c** was prepared from **4** with 48% yield as reference for the latter carbon-11-labeling. In case of the introduction of both the PMB and the methyl group, the respective 2*H*-regioisomer was obtained supplementary.[30] No 2*H*-regioisomer was found when applying the EOE group.

Next, the nitro group of compounds **4** and **5a–c** was reduced with H_2/Pd–C to obtain amines **6a–d**. The resulting amino group attached to position 4 of the indazole moiety is necessary for the following introduction of the pyrimidine residue. Thus, **6a–d** were reacted with 2,4-dichloropyrimidine in absolute ethanol and DIPEA to obtain **7a–d** in yields from 17% to 57%. The last step afforded the methylation of the secondary amine. Therefore, **7a–c** were treated with methyl iodide to give **8a–c** in high yields of 88–91%. The reaction path and the conditions were figured out in Scheme 3.

The molecular structure of EOE-protected compound **5b** was validated independently by a single-crystal X-ray analysis and the resulting molecular structure of **5b** is shown in Figure 4.[31]

Next, 3,5-di(morpholino)aniline **9** as second substructure (part B) was synthesized from the respective nitro compound as pointed out in the literature. [23,32] Crystals of **9** suitable for a single crystal X-ray analysis were grown from a saturated ethyl acetate/petroleum ether solution and the molecular structure was expressed in Figure 5.[31]

Finally, two reactions paths were figured out for the connection of substructures A (**7a–d** and **8a–c**) with B (compound: **9**). In the first path, the connection reaction was done under acid catalysis. Thus, compounds **7a–d** and **8a** were dissolved in anhydrous dioxane and stirred at 90 °C overnight. The desired products **10**, **11**, **14** and **16** were obtained in good yields (44–83%) except for **7b** which led to deprotected derivative **16**. Hereupon, it was necessary that both EOE protected substances **7b** and **8b** were reacted with **9** in a Pd-catalyzed Buchwald–Hartwig amination. In addition, **7c** and **8c** were reacted as well using the same conditions and the resulting compounds **12–15** were obtained in similar yields (62–83%) compared to the acid catalyzed reaction path A. All results were pointed out in Scheme 4 and Table 2. The final step requires the removal of the protecting groups. Unfortunately, all our attempts to eliminate the PMB protecting group from **10** and **11** failed (see later in detail). Based on these results, the EOE group is exposed to be the better choice for this purpose and was applied instead for this protection purpose. In this context, original inhibitor **17**, which serves as reference for the radiolabeling with [^{11}C]methyl



Scheme 3. Synthesis path for substructure A. Reagents and conditions: (a) 4-methoxybenzyl chloride, Cs_2CO_3, DMF, 110 °C, 2 h; (b) ethyl vinyl ether, *p*-TsOH, rt, 1 h; (c) MeI, NaH, 0 °C, 1.5 h; (d) Pd/C-H_2, MeOH–CH_2Cl_2, rt, 1 d; (e) 2,4-dichloropyrimidine, DIPEA, EtOH, 90 °C, 2 d; (f) MeI, Cs_2CO_3, DMF, rt, 4 h.

iodide, was obtained when **13** was deprotected with 1 M HCl at ambient temperature or with *p*-TsOH at 100 °C for 24 h in a high yield of 92%.

Two positions of the original inhibitor molecule **IV** were envisaged for the incorporation of fluorine-18/19. Optimally, the methyl group of the original inhibitor **IV** was replaced by the 3-fluoropropyl moiety. Thus, compound **7b** was treated with NaH for 30 min. Afterwards, 1-fluoro-3-iodopropane was added and **18a** (98%) was yielded after aqueous workup. Next, the *p*-TsOH catalyzed reaction with aniline **9** was accomplished to obtain the EOE protected fluorine-19 containing reference compound **19a**. After the workup, the EOE protecting group was removed immediately using 1 M HCl and reference compound **20** was achieved in 59% yield.

Further, the respective precursor was synthesized as follows. In the presence of Cs_2CO_3 as base, compound **7b** was treated with 3-iodopropanol which was previously prepared from 3-bromopropanol and NaI in a Finkelstein exchange reaction. In the next step, **18b** was reacted with aniline **9** under Buchwald–Hartwig conditions to give **19b**. However, under these conditions, starting material was still present. A complete conversion of **9** was not observed. To fix this problem, the *p*-TsOH catalyzed reaction with aniline **9** was accomplished to obtain the EOE protected hydroxypropyl compound **19b**. Next, **19b** was first tried to tosylate with *p*-TsCl and Et_3N as base to yield precursor **21a**, however, no isolatable product was obtained. As an alternative, mesyl chloride was used with DIPEA as mild base at ambient temperature; however, the open-chained compound **21b** was also not obtainable. Instead the azoniaspiro compound **22** was isolated in 83% yield, but no signal was found for the mesyl group in the ^{1}H NMR analysis. Thus, the solvent of the crude reaction mixture of **22** was removed and the residue was immediately dissolved in acetonitrile. Silver mesylate was added and the mixture was stirred for 30 min in the dark. After changing the solvent to dichloromethane and filtration of the precipitated silver salts, the final spiro precursor **23** was yielded after purification via column chromatography. The full reaction sequence was pointed out in Scheme 5.

2.3. Carbon-11 radiolabeling

Carbon-11 enables the radiolabeling of bioactive molecules without changing the chemical structure and the biochemical properties due to the isotopic exchange. The most commonly applied labeling method consists of the [^{11}C]methylation using [^{11}C]CH_3I or [^{11}C]CH_3OTf.[33,34] The original inhibitor contains an amine bound methyl group which is excellently suitable for the isotopic labeling with carbon-11. In first experiments, the optimal labeling conditions for the literature known compound **17** was investigated. Problematic, three secondary amino functions are present in this starting molecule feasible to be alkylated. Prior to the radiolabeling, it was evaluated, which of the three amino functions in precursor **16** are preferred to be methylated. For this purpose, **16** was treated with 1 equiv of methyl iodide. In addition to the desired original inhibitor **17** (61% yield) which serves as reference, the twice alkylated compound **15** was yielded (14%). However, the threefold methylated product was not found even when treated with a higher excess of MeI. Therefore, it was necessary to develop a protecting strategy only for the indazole moiety to avoid the introduction of the methyl group at this place and in this case a twofold labeling of **16** with [^{11}C]CH_3I. As indicated previously in Scheme 3, the *p*-methoxybenzyl group and the ethoxyethyl group were applied for the protection of the indazole ring. Both groups are stable under strong basic conditions. Thus, it should be possible to remove these PGs either with mineral acids (EOE),[29] with DDQ (PMB)[35] or with strong acids like TFA (EOE/PMB).[36] However, preliminary inquiries pointed out that it was impossible to cleave the PMB group with DDQ as well as with TFA under different conditions.

Based on these results, the EOE protected compound **12** (t_R = 8.6 min) was chosen as precursor for the introduction of [^{11}C]CH_3I. The labeling reaction proceeded in two steps. In a first experiment, **12** was treated with 0.5 M NaOH followed by alkylation with [^{11}C]CH_3I leading to [^{11}C]**13** (t_R = 11.1 min). In a next experiment, subsequent deprotection of the EOE group with 1 M HCl solution (200 µL) for 2 min at 60 °C was executed to yield [^{11}C]**17** (t_R = 5.8 min). Optimized conditions were found to be approx. 1.2 mg of precursor **12** under basic conditions (40 µL of 0.5 M NaOH) for 2 min at 80 °C. Unknown by-products were found at t_R = 2.5–3.5 min and residual [^{11}C]CH_3I at t_R = 9.25 min which can be separated via semi-preparative HPLC purification. The results are summarized in Scheme 6 and selected HPLC

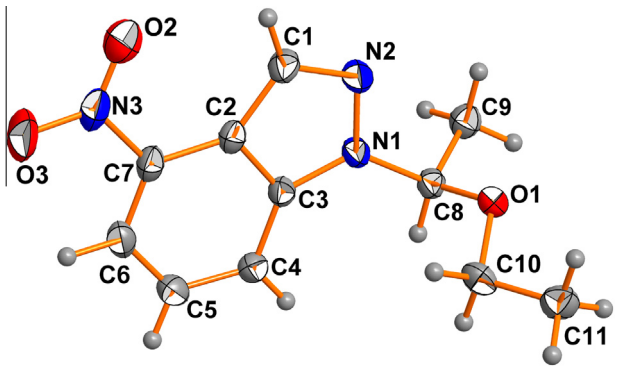


Figure 4. ORTEP presentation of the molecular structure of **5b** with the displacement ellipsoids drawn at the 50% probability level.

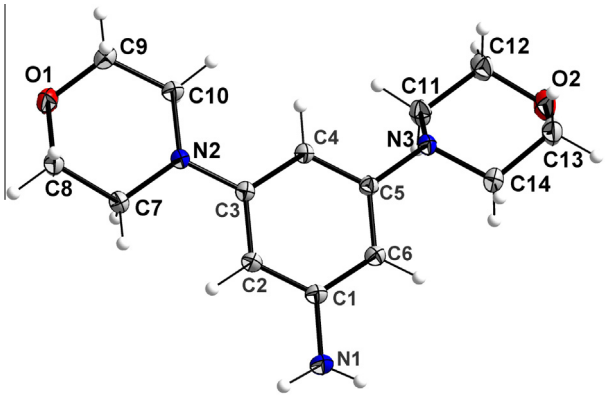
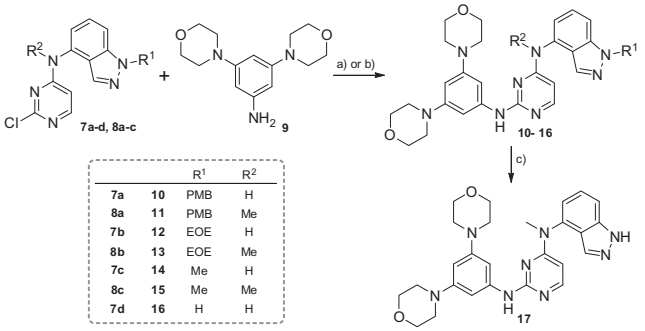
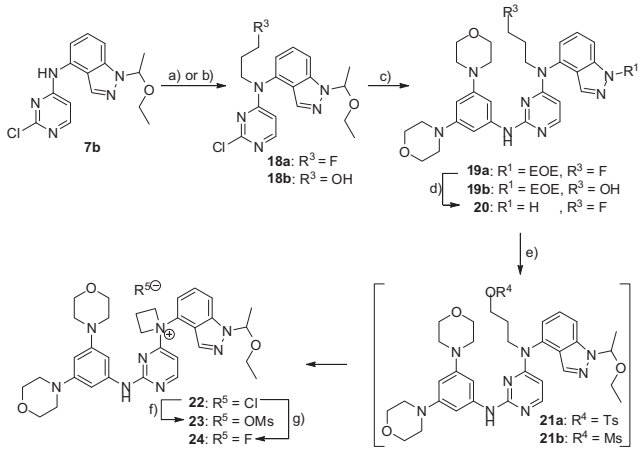


Figure 5. ORTEP presentation of the molecular structure of **9** with the displacement ellipsoids drawn at the 50% probability level.



Scheme 4. Synthesis path for precursors and reference compound **17**. Reagents and conditions: (a) *p*-TsOH, dioxane, 90 °C, overnight; (b) Cs_2CO_3, Xantphos, Pd_2dba_3, dioxane, 100 °C, overnight; (c) HCl, EtOH, 1.5 h.



Scheme 5. Synthesis path for precursor **23** and reference compound **20**. Reagents and conditions: (a) 1-fluoro-3-iodopropane, NaH, DMF, 80 °C, overnight; (b) 3-bromopropanol, NaI, acetone, rt, overnight then Cs_2CO_3, DMF, 80 °C, overnight; (c) **9**, *p*-TsOH, dioxane, 90 °C, overnight; (d) HCl, 1.5 h; (e) MsCl, DIPEA, DCM, 0 °C, 1 h; (f) AgOMs, ACN, 1 h, rt, dark; (g) AgF, ACN, 1 h, rt, dark.

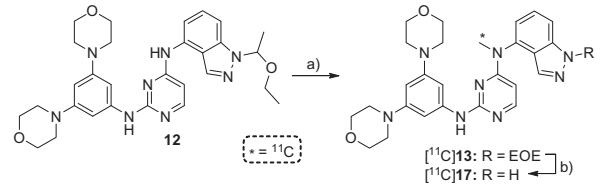
chromatograms were shown in Figure 6. In a typical experiment, [^{11}C]**17** was obtained after purification via radio-HPLC in a RCY of 30–35% (d.c.) within a synthesis time of 20 min (after EOB) starting from approx. 1–1.5 GBq [^{11}C]CH_3I.

2.4. Fluorine-18 radiolabeling

The introduction of radiofluorine should be realized by the nucleophilic displacement with [^{18}F]fluoride. For this purpose, the protic hydrogen of the indazole moiety in the original inhibitor was blocked with the EOE protecting group. The other secondary amine should not influence the radiolabeling as it was shown by us recently.[26] Furthermore, we could demonstrate that precursors which contain an azoniaspirononane moiety are highly suitable in case of the nucleophilic introduction of [^{18}F]F$^-$ in excellent radiochemical yields and in case of the latter purification.[37,38]

In the first step, EOE-precursor **23** was treated with [^{18}F]fluoride in acetonitrile at 100 °C for 30 min. HPLC analyses showed a signal at t_R = 3.0 min (γ-trace) which did not belong to the non-radioactive EOE reference compound **19a** (t_R = 13.5 min, UV trace). After cleavage of the EOE group with 2 M HCl a signal of an unknown species at t_R = 7 min appeared which did not belong to ^{19}F-reference **20**. This result was also obtained after changing the solvent (DMF, DMSO), rising the temperature (100–150 °C) and elongating the reaction time (up to 2 h). This could indicate, that the [^{18}F]fluorine is not covalently bound to the rest of the molecule. In addition, free [^{18}F]fluoride was still found as shown by radio-TLC analyses. It is more likely, that compound [^{18}F]**24** appeared instead of [^{18}F]**20** having non-covalently bound [^{18}F]fluorine as [^{18}F]fluoride. A possible explanation for this indication could be that the formed spiro ammonium moiety of precursor **20** between the two aromatic rings is highly stable and the positive charge is delocalized. In this case, a nucleophilic attack of the neighboring carbon atom of the four-membered ring is not possible.

In order to prove and verify this, **22** was treated with AgF in acetonitrile to change chloride to fluoride (see Scheme 5). The ^{19}F NMR of resulting derivative **24** (yield: 27%) showed a singlet at



Scheme 6. Radiolabeling of **12** with [^{11}C]CH_3I followed by deprotection with HCl to yield [^{11}C]**17**. Reagents and conditions: (a) [^{11}C]MeI, NaOH, DMF, 80 °C, 2 min; (b) HCl, DMF, 80 °C, 2 min.

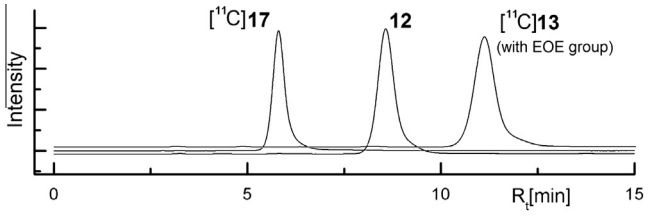
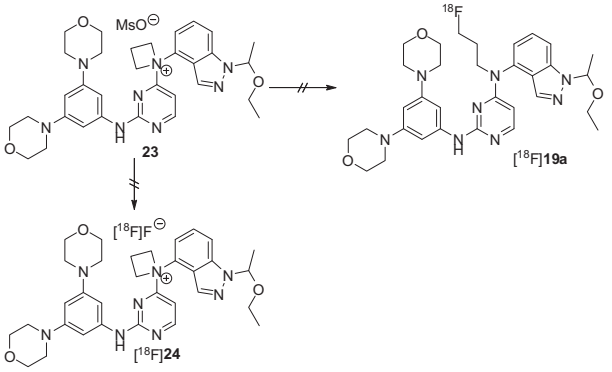


Figure 6. (Radio-)HPLC chromatograms of **12** (UV trace), [^{11}C]**13** (γ trace), and [^{11}C]**17** (γ trace).



Scheme 7. Possible results from [^{18}F]radiolabeling of precursor **23**.

Table 2
Scope of the connection of different substructures A with **9**

Entry	Educt	R^1	R^2	Path	Product	Yield (%)
1	**7a**	PMB	H	**A**	**10**	78
2	**8a**	PMB	Me	**A**	**11**	44
3	**7b**	EOE	H	**(A) B**	**(16) 12**	(21) 73
4	**8b**	EOE	Me	**B**	**13**	62
5	**7c**	Me	H	(**A**) **B**	**14**	(73) 83
6	**8c**	Me	Me	**B**	**15**	80
7	**7d**	H	H	**A**	**16**	79

−120 ppm (compared to −220.0 ppm of **20**) and no coupling to protons was observed. This was furthermore confirmed by the analysis of the ^{1}H and ^{13}C NMR spectra. This finding evidences, that the fluorine is not covalently bound in **24**. In fact, the spiro ammonium cation with the appropriate fluoride as anion was formed. Additionally, only a peak at $m/z = 585$ which belongs to the spiro compound was found in MS analyses of **24** (compared to $m/z = 605$ for **19a** or $m/z = 533$ for **20**, both with covalently bound fluorine). Based on these results, compound **24** was also analyzed by HPLC. A comparison of the t_R-values clearly pointed out that **24** does not belong to the above described unknown peak in the γ-trace, too. Thus, at this stage it is not possible to prepare [^{18}F]**20** as [^{18}F]radiotracer using this method (Scheme 7).

3. Conclusion

A successful synthesis of precursors as well as reference compounds for ^{11}C-/^{18}F-radiolabeling based on a highly affine EphB4 receptor inhibitor is demonstrated. As a prerequisite, docking studies were accomplished pointing out the preferred position in the original molecule for the introduction of a 3-[^{18}F]fluoropropyl moiety which was not mandatory for the isotopic labeling with carbon-11. For radiolabeling purposes, a convenient protecting group strategy was established based on the application of the ethoxyethyl (EOE) group. Radiolabeling with carbon-11 was successfully accomplished leading to the isotopically labeled radiotracer whereas the radiofluorination with [^{18}F]fluoride did not lead to the desired radiotracer under the chosen conditions. Thus, our ongoing research is focused on an alternative preparation of the [^{18}F]radiotracer based on this lead structure and on the (radio)biological evaluation of the [^{11}C]radiotracer.

4. Experimental

4.1. General

All reagents were purchased from commercial suppliers and were used without further purification. Analytical TLC was performed on pre-coated Silica Gel 60 F_{254} plates (Merck) and results read under UV-light ($\lambda = 254$ nm). ^{1}H NMR, ^{13}C NMR as well as ^{19}F NMR spectra were recorded on a Varian Inova-400 or on an Agilent DD2-400 (OneNMR Probe) spectrometer at 400, 101, and 376 MHz, respectively. Chemical shifts are reported in ppm with tetramethylsilane (^{1}H, ^{13}C) and trichlorofluoromethane (^{19}F) as internal standard, respectively. Mass spectrometric (MS) data were obtained on a Quattro/LC mass spectrometer (MICROMASS) or on a Xevo TQ-S (Waters) by electron spray ionization (ESI). Melting points were recorded on a Cambridge Instruments Galen III apparatus and are uncorrected. Diffraction data of **5b** and **9** were collected with a Bruker-Nonius Apex-X8 CCD-diffractometer using graphite-monochromated Mo–K_α radiation ($\lambda = 0.71073$ Å). The diffraction measurement was done at −100 °C and the structures were solved by Direct Methods using Shelxs-97 and refined against F^2 on all data by full-matrix least-squares with Shelxl-97.[39] All non-hydrogen atoms were refined anisotropically; all hydrogen atoms bonded to C atoms were placed on calculated positions and refined using a riding model. Analytical HPLC was performed on a VWR/Hitachi Elite La Chrome HPLC system, equipped with a reverse phase column (Nucleosil 100-5C18 Nautilus), a UV-diode array detector (254 nm) and a scintillation radiodetector (Raytest, Gabi Star) at a flow rate of 1 mL/min (eluent: acetonitrile/water, 30:70 + 0.1% TFA). The radioactive compound was identified with analytical radio-HPLC by comparison of the retention time of the reference compound. Decay-corrected RCYs were quantified by integration of radioactive peaks on a radio-TLC using a radio-TLC scanner (Fuji, BAS2000). [^{18}F]Fluoride and [^{11}C]CH_4 were obtained utilizing the PET cyclotron Cyclone 18/9 (IBA, Belgium). [^{18}F]Fluoride was produced by the $^{18}O(p,n)^{18}F$ nuclear reaction, [^{18}O]H_2O was irradiated. [^{11}C]CH_4 was produced by the $^{14}N(p,\alpha)^{11}C$ nuclear reaction by irradiation of N_2 gas containing 10% H_2 using an aluminum target. Radiosyntheses were performed in an automated nucleophilic synthesizer TracerLab$_{FxC}$ (GE) that was modified in terms of direct trapping of [^{11}C]CH_4.

4.2. Synthesis

Compounds **4**, **5a**, **5c**, **6c**, **6d**, **7d**, **9**, **15** and **16** were prepared according to the literature. Additional details and procedures can be found in the Supporting information.

4.2.1. 1-(1-Ethoxyethyl)-4-nitro-1*H*-indazole (5b)

Ethyl vinyl ether (3.978 g, 35.17 mmol) was added to a solution of 4-nitro-1*H*-indazole (**4**) (3 g, 18.39 mmol) and *p*-toluene sulfonic acid (350 mg, 1.839 mmol) in 50 mL anhydrous dichloromethane. After stirring at rt for 1 h, the reaction mixture was quenched with water (20 mL) and saturated bicarbonate solution (20 mL). After stirring for 30 min, the aqueous layer was extracted with dichloromethane (3 × 25 mL), the combined organic layers were dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification was done by column chromatography (PE–EtOAc = 20:1) to yield compound **5b** (3.4 g, 80%) as yellow solid. Mp 58 °C; $R_f = 0.54$ (PE–EtOAc = 1:1); δ_H (400 MHz, $CDCl_3$) 1.14 (3H, t, $^{3}J = 7.1$, CH_3CH_2), 1.81 (3H, $^{3}J = 6.1$, CH_3CH), 3.28 (1H, dq, $^{2}J = 9.2$, $^{3}J = 7.1$, CH_2), 3.49 (1H, dq, $^{2}J = 9.2$, $^{3}J = 7.1$, CH_2), 5.97 (1H, q, $^{3}J = 6.1$, CH), 7.51 (1H, dd, $^{3}J = 7.8$, $^{3}J = 8.4$, 6-H), 8.12 (1H, d, $^{3}J = 8.4$, 5-H), 8.17 (1H, d, $^{3}J = 7.8$, 7-H), 8.52 (1H, s, 3-H); δ_C (101 MHz, $CDCl_3$) 14.9, 21.2 (2× CH_3), 64.2 (CH_2), 88.1 (CH), 117.9 (C-7), 118.2 (C-3a), 118.6 (C-5), 125.6 (C-6), 132.8 (C-3), 139.9 (C-7a), 140.8 (C-4); MS (ESI$^-$): m/z 235 (100, M^+); elemental analysis calcd for $C_{11}H_{13}N_3O_3$ (235.24): C, 56.16, H, 5.57, N, 17.86; found: C, 56.44, H, 5.72, N, 16.87.

4.2.2. 4-Amino-1-(4-methoxybenzyl)-1*H*-indazole (6a)

Compound **5a** (875 mg, 3.08 mmol) was dissolved in methanol/dichloromethane (30 mL, v:v = 1:1), treated with Pd/C (500 mg) and stirred at rt and 1 bar H_2 for 24 h. Subsequently, the catalyst was filtered and the solvent was removed under reduced pressure. Purification was done by column chromatography (PE–EtOAc = 1:1 → 1:2) to yield compound **6a** (697 mg, 89%) as a brown solid. Mp 99 °C; $R_f = 0.31$ (PE–EtOAc = 1:1). δ_H (400 MHz, $CDCl_3$) 3.75 (3H, s, CH_3), 5.47 (2H, s, CH_2), 6.31 (1H, d, $^{3}J_{5,6} = 7.5$, 5-H), 6.77 (1H, d, $^{3}J_{7,6} = 8.3$, 7-H), 6.82 (2H, dd, $^{3}J = 8.6$, H-o), 7.12 (1H, dd, $^{3}J_{5,6} = 7.5$, $^{3}J_{7,6} = 8.3$, 6-H), 7.16 (2H, dd, $^{3}J = 8.6$, H-m), 7.97 (1H, s, 3-H); δ_C (101 MHz, $CDCl_3$): $\delta = 52.7$ (CH_3), 55.4 (CH_2), 99.6 (C-7), 103.8 (C-5), 114.3 (C-m), 115.2 (C-3a), 128.1 (C-6), 128.8 (C-o), 129.3 (C-i), 130.5 (C-3), 140.5 (C-7a), 159.3 (C-p); MS (ESI$^+$): m/z 254 (100, M^++H); elemental analysis calcd for $C_{15}H_{15}N_3O$ (253.30): C, 71.13, H, 5.97, N, 16.59; found: C, 71.43, H, 6.06, N, 16.52.

4.2.3. 1-(1-Ethoxyethyl)-1*H*-indazol-4-amine (6b)

Compound **5b** (3.4 g, 14.45 mmol) was dissolved in methanol/dichloromethane (30 mL, v:v = 1:1) and Pd/C (500 mg) was added. This mixture was treated with H_2 (approx. 1 bar) at rt for 24 h. Subsequently, the catalyst was filtered and the solvent was removed under reduced pressure. Purification was done by column chromatography (PE–EtOAc = 2:1) to yield compound **6b** (1.22 g, 41%) as a brown oil. $R_f = 0.58$ (PE–EtOAc = 2:1); δ_H (400 MHz, $CDCl_3$) 1.12 (3H, t, $^{3}J = 7.0$, CH_3CH_2), 1.78 (3H, d, $^{3}J = 6.2$, CH_3CH), 3.25 (1H, dq, $^{2}J = 9.3$, $^{3}J = 7.0$, CH_2), 3.43 (1H, dq, $^{2}J = 9.3$, $^{3}J = 7.0$, CH_2), 5.84 (1H, q, $^{3}J = 6.2$, CH), 6.36 (1H, d, $^{3}J = 7.4$ Hz, 5-H), 7.06

(1H, d, ^3J = 8.4, 7-H), 7.17 (1H, dd, ^3J = 8.4, ^3J = 7.4, 6-H), 7.94 (1H, s, 3-H); δ_C (101 MHz, $CDCl_3$) 14.9, 21.2 (2× CH_3), 64.2 (CH_2), 88.1 (CH), 117.9 (C-7), 118.2 (C-3a), 118.6 (C-5), 125.6 (C-6), 132.8 (C-3), 139.9 (C-7a), 140.8 (C-4); MS (ESI$^+$): *m*/*z* 228 (20, M$^+$+Na), 206 (100, M$^+$+H); elemental analysis calcd for $C_{11}H_{15}N_3O$ (205.26): C, 64.37, H, 7.37, N, 20.47; found: C, 63.92, H, 7.20, N, 19.35.

4.2.4. *N*-(2-Chloropyrimidin-4-yl)-1-(4-methoxybenzyl)-1*H*-indazol-4-amine (7a)

2,4-Dichloropyrimidine (156 mg, 1.045 mmol) and DIPEA (142 mg, 1.095 mmol) were added to **6a** (252 mg, 0.995 mmol) dissolved in absolute ethanol (5 mL). This mixture was allowed to stir under reflux for 2 d. After cooling to rt, water (5 mL) was added, the aqueous layer was extracted with EtOAc (3 × 10 mL). The combined organic layers were dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification was done by column chromatography (PE–EtOAc = 2:1 → 1:1) to yield compound **7a** (207 mg, 57%) as light red solid. Mp 67 °C; R_f = 0.45 (PE–EtOAc = 1:2); δ_H (400 MHz, $CDCl_3$) 3.77 (3H, s, CH_3), 5.55 (2H, s, CH_2), 6.65 (1H, d, ^3J = 5.9, 5″-H), 6.84 (2H, dd, ^3J = 8.7, H-m), 7.16 (1H, d, ^3J = 7.4, 5-H), 7.19 (2H, dd, ^3J = 8.7, H-o), 7.29 (1H, d, ^3J = 8.4, 7-H) 7.35 (1H, dd, ^3J = 7.4, ^3J = 8.4, 6-H), 7.96 (1H, s, 3-H), 8.15 (1H, d, ^3J = 5.9, 6″-H); δ_C (101 MHz, $CDCl_3$) 53.2 (CH_3), 55.5 (CH_2), 103.0 (C-5″), 107.8 (C-7), 114.4 (C-m), 120.0 (C-3a), 127.2 (C-6), 128.5 (C-1″), 129.0 (C-o), 129.9 (C-4), 130.7 (C-3), 140.9 (C-7a), 158.3 (C-6″), 158.4 (C-p), 159.5 (C-2″), 162.6 (C-4″); MS (ESI$^-$): *m*/*z* 366 (40, M$^+$−H, ^{37}Cl), 364 (100, M$^+$−H, ^{35}Cl); elemental analysis calcd for $C_{19}H_{16}N_5OCl$ (365.82): C, 62.38, H, 4.41, N, 19.14; found: C, 62.62, H, 4.52, N, 18.73.

4.2.5. *N*-(2-Chloropyrimidin-4-yl)-1-(1-ethoxyethyl)-1*H*-indazol-4-amine (7b)

2,4-Dichloropyrimidine (1.51 g, 10.14 mmol) and DIPEA (1.06 g, 8.19 mmol) were added to **6b** (1.6 g, 7.80 mmol) dissolved in absolute ethanol (25 mL). This mixture was allowed to stir under reflux for 2 d. After cooling to rt, water (20 mL) and 10% $NaHSO_4$ solution (2 mL) was added, the aqueous layer was extracted with EtOAc (3 × 20 mL). The combined organic layers were dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification was done by column chromatography (PE–EtOAc = 2:1 → 1:1) to yield compound **7b** (1.3 g, 52%) as colorless solid. Mp 160 °C; R_f = 0.39 (PE–EtOAc = 1:2); δ_H (400 MHz, $CDCl_3$) 1.15 (3H, t, ^3J = 7.1, C*H*$_3$CH$_2$), 1.81 (3H, d, ^3J = 6.1, C*H*$_3$CH), 3.28 (1H, dq, ^2J = 9.2, ^3J = 7.1, CH_2), 3.48 (1H, dq, ^2J = 9.2, ^3J = 7.1, CH_2), 5.91 (1H, q, ^3J = 6.1, CH), 6.65 (1H, d, ^3J = 5.9, 5′-H), 7.20 (1H, d, ^3J = 7.4, 5-H), 7.27 (1H, s, NH), 7.40 (1H, dd, ^3J = 7.4, ^3J = 8.5, 6-H), 7.62 (1H, d, ^3J = 8.5, 7-H), 7.93 (1H, s, 3-H), 8.17 (1H, d, ^3J = 5.9, 6′-H); δ_C (101 MHz, $CDCl_3$) 15.0, 21.1 (2× CH_3), 64.1 (CH_2), 87.5 (CH), 102.9 (C-7), 109.1 (C-5′), 114.9 (C-5), 120.4 (C-3a), 127.3 (C-6), 130.0 (C-3), 130.7 (C-4), 140.0 (C-7a), 158.7 (C-2′), 161.1 (C-6′), 162.6 (C-4′). MS (ESI$^+$): *m*/*z* 342 (10, M$^+$+Na, ^{37}Cl), 340 (20, M$^+$+Na, ^{35}Cl), 320 (32, M$^+$+H, ^{37}Cl), 318 (100, M$^+$+H, ^{35}Cl); elemental analysis calcd for $C_{15}H_{16}N_5OCl$ (317.77): C, 56.69, H, 5.08, N, 22.04; found: C, 57.12, H, 5.25, N, 21.53.

4.2.6. 4-Amino-*N*-(2-chloropyrimidin-4-yl)-1-methyl-1*H*-indazole (7c)

2,4-Dichloropyrimidine (1.34 g, 9.02 mmol) and DIPEA (1.22 g, 9.45 mmol) were added to **6c** (1.26 g, 8.59 mmol) dissolved in absolute ethanol (25 mL). This mixture was allowed to stir under reflux for 2 d. After cooling to rt, water (20 mL) was added, the aqueous layer was extracted with EtOAc (3 × 25 mL). The combined organic layers were dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification was done by column chromatography (PE–EtOAc = 2:1 → 1:1) to yield compound **7c** (1.054 g, 47%) as light red solid. Mp 169 °C; R_f = 0.44 (PE–EtOAc–EtOH = 2:6:1); δ_H (400 MHz, $CDCl_3$) 4.11 (3H, s, CH_3), 6.62 (1H, d, ^3J = 5.9, H-5′), 7.19 (1H, d, ^3J = 7.5, 5-H), 7.31 (1H, d, ^3J = 8.6, 7-H), 7.41 (1H, dd, ^3J = 8.6, ^3J = 7.5, 6-H), 7.91 (1H, d, ^4J = 0.7, 3-H), 8.14 (1H, d, ^3J = 5.9, 6′-H); δ_C (101 MHz, $CDCl_3$) 35.7 (CH_3), 102.7 (C-7), 107.0 (C-5′), 114.1 (C-5), 118.9 (C-3a), 126.8 (C-6), 129.6 (C-3), 129.9 (C-4) 141.1 (C-7a), 158.1 (C-2′), 160.7 (C-6′), 162.4 (C-4′); MS (ESI$^+$): *m*/*z* 262 (20, M$^+$+H, ^{37}Cl), 260 (58, M$^+$+H, ^{35}Cl); elemental analysis calcd for $C_{12}H_{10}ClN_5$ (259.69): C, 55.50, H, 3.88, N, 26.97; found: C, 54.97, H, 4.00, N, 26.63.

4.2.7. *N*-(2-Chloropyrimidin-4-yl)-1-(4-methoxybenzyl)-*N*-methyl-1*H*-indazol-4-amine (8a)

Cs_2CO_3 (179 mg, 0.548 mmol) and MeI (29 mg, 0.205 mmol) were added to **7a** (50 mg, 0.137 mmol) dissolved in anhydrous DMF (3 mL) at 0 °C. Then, the solution was stirred at rt for 4 h. Afterwards, water (15 mL) was added and the aqueous layer was extracted with EtOAc (3 × 15 mL). The combined organic layers were dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification was done by column chromatography (PE–EtOAc = 1:1) to yield compound **8a** (50 mg, 96%) as pale yellow syrup. R_f = 0.55 (PE–EtOAc–EtOH = 2:6:1); δ_H (400 MHz, $CDCl_3$) 3.59 (1H, s, NCH_3), 3.77 (1H, s, OCH_3), 5.55 (2H, s, CH_2), 6.06 (1H, d, ^3J = 6.0, H-5″), 6.85 (2H, d, ^3J = 8.5, H-o), 7.01 (1H, t, ^3J = 4.8, ^3J = 3.4, 6-H), 7.23 (2H, d, ^3J = 8.5, H-m), 7.39–7.40 (2H, m, 5/7-H), 7.80 (1H, s, 3-H), 7.84 (1H, d, ^3J = 6.0, 6″-H); δ_C (101 MHz, $CDCl_3$) 38.2, 53.2 (2× CH_3), 55.4 (CH_2), 103.9 (C-7), 109.6 (C-5″), 114.3 (C-o), 118.8 (C-5), 121.1 (C-3a), 127.4 (C-6), 128.4 (C-1′), 129.0 (C-m), 131.0 (C-3), 136.2 (C-4), 141.1 (C-7a), 156.4 (C-6″), 159.5 (C-4′), 160.8 (C-2″), 163.5 (C-4″); MS (ESI$^+$): *m*/*z* 382 (28, M$^+$+H, ^{37}Cl), 380 (70, M$^+$+H, ^{35}Cl); elemental analysis calcd for $C_{19}H_{16}N_5OCl$ (365.82): C, 62.38, H, 4.41, N, 19.14; found: C, 62.62, H, 4.52, N, 18.73.

4.2.8. *N*-(2-Chloropyrimidin-4-yl)-1-(1-ethoxyethyl)-*N*-methyl-1*H*-indazol-4-amine (8b)

Cs_2CO_3 (615 mg, 1.887 mmol) and MeI (134 mg, 0.944 mmol) were added to **7b** (200 mg, 0.629 mmol) dissolved in anhydrous DMF (5 mL) at 0 °C. Then, the solution was stirred at rt for 4 h. Afterwards, water (15 mL) was added and the aqueous layer was extracted with EtOAc (3 × 15 mL). The combined organic layers were dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification was done by column chromatography (PE–EtOAc = 1:1) to yield compound **8b** (183 mg, 88%) as colorless solid. Mp 105 °C. R_f = 0.26 (PE–EtOAc = 2:1). δ_H (400 MHz, $CDCl_3$) 1.14 (3H, t, ^3J = 7.1, C*H*$_3$CH$_2$), 1.78 (3H, d, ^3J = 6.1, C*H*$_3$CH), 3.29 (1H, dq, ^2J = 9.3, ^3J = 7.1, CH_2), 3.47 (1H, dq, ^2J = 9.3, ^3J = 7.1, CH_2), 3.58 (3H, s, CH_3), 5.90 (1H, q, ^3J = 6.1, CH), 6.07 (1H, d, ^3J = 6.0, 5′-H), 7.04 (1H, d, ^3J = 7.4, 5-H), 7.42 (1H, dd, ^3J = 7.4, ^3J = 8.5, 6-H), 7.72 (1H, d, ^3J = 8.5, 7-H), 7.76 (1H, s, 3-H), 7.84 (1H, d, ^3J = 6.0, 6′-H); δ_C (101 MHz, $CDCl_3$) 14.9, 21.1, 38.2 (3× CH_3), 64.1 (CH_2), 87.5 (CH), 103.8 (C-7), 110.8 (C-5′), 119.2 (C-5), 121.7 (C-3a), 127.4 (C-6), 131.0 (C-3), 136.2 (C-4), 140.1 (C-7a), 156.4 (C-6′), 160.8 (C-2′), 163.5 (C-4′); MS (ESI$^+$): *m*/*z* 334 (35, M$^+$+H, ^{37}Cl), 332 (100, M$^+$+H, ^{35}Cl),; elemental analysis calcd for $C_{16}H_{18}N_5OCl$ (331.80): C, 57.92, H, 5.47, N, 21.11; found: C, 58.28, H, 5.54, N, 20.78.

4.2.9. *N*-(2-Chloropyrimidin-4-yl)-*N*,1-dimethyl-1*H*-indazol-4-amine (8c)

Cs_2CO_3 (655 mg, 2.01 mmol) and MeI (142 mg, 1.00 mmol) were added to **7c** (174 mg, 0.67 mmol) dissolved in anhydrous DMF (5 mL) at 0 °C. Then, the solution was stirred at rt for 4 h. Afterwards, water (15 mL) was added and the aqueous layer was extracted with EtOAc (3 × 15 mL). The combined organic layers were dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification was done by column

chromatography (PE–EtOAc = 1:1) to yield compound **8c** (166 mg, 91%) as pale yellow solid. Mp 142 °C; R_f = 0.55 (PE–EtOAc–EtOH = 2:6:1); δ_H (400 MHz, $CDCl_3$) 3.58 (3H, s, NCH_3), 4.11 (3H, s, CH_3), 6.03 (1H, d, ^3J = 6.1, 5′-H), 7.02 (1H, dd, ^3J = 6.5, ^3J = 7.9, 6-H), 7.41 (1H, d, ^3J = 6.5, 5-H), 7.44 (1H, d, ^3J = 7.9, 7-H), 7.74 (1H, s, 3-H), 7.82 (1H, d, ^3J = 6.1, 6′-H); δ_C (101 MHz, $CDCl_3$) 36.0, 38.1 (2× CH_3), 103.8 (C-7), 109.1 (C-5′), 118.7 (C-5), 120.7 (C-3a), 127.3 (C-6), 130.5 (C-3), 136.1 (C-4), 141.7 (C-7a), 156.4 (C-6′), 160.8 (C-2′), 163.5 (C-4′); MS (ESI$^+$) *m*/*z* 276 (35, M^++H, ^{37}Cl), 274 (100, M^++H, ^{35}Cl); elemental analysis calcd for $C_{13}H_{12}N_5Cl$ (273.72): C, 57.04, H, 4.42, N, 25.59; found: C, 57.51, H, 4.51, N, 25.30.

4.2.10. N^2-(3,5-Dimorpholinophenyl)-N^4-(1-(4-methoxybenzyl)-1*H*-indazol-4-yl)pyrimidin-2,4-diamine (10)

Compound **7a** (189 mg, 0.516 mmol) and compound **9** (150 mg, 0.568 mmol) were dissolved in anhydrous dioxane (5 mL) and *p*-TsOH in catalytic amounts was added. Afterwards, the reaction mixture was stirred at 90 °C for 1 d. The reaction was quenched with water (15 mL) and half-saturated bicarbonate solution (4 mL). The aqueous layer was extracted with EtOAc (3 × 15 mL). The combined organic layers were dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification was done by column chromatography ($CHCl_3$–MeOH = 98:2) to yield compound **10** (239 mg, 78%) as light brown solid. Mp 122 °C; R_f = 0.69 ($CHCl_3$–MeOH = 95:5); δ_H (400 MHz, $CDCl_3$) 3.07 (8H, t, ^3J = 4.7, CH_2N), 3.74 (3H, s, CH_3), 3.77 (8H, t, ^3J = 4.7, CH_2O), 5.49 (2H, s, CH_2), 6.13 (1H, t, ^4J = 1.9, 4‴-H), 6.21 (1H, d, ^3J = 5.8, 5-″H), 6.75 (2H, d, ^4J = 1.9, 2‴/6‴-H), 6.81 (2H, d, ^3J = 8.6, H-o), 7.13 (1H, d, ^3J = 8.3, 5-H), 7.15 (2H, d, ^3J = 8.6, H-m), 7.27 (1H, dd, ^3J = 7.6 Hz, ^3J = 8.3, 6-H), 7.33 (1H, d, ^3J = 7.6, 7-H), 7.53 (1H, s, NH), 7.55 (1H, s, NH), 7.96 (1H, s, 3-H), 8.04 (1H, d, ^3J = 5.8, 6″-H); δ_C (101 MHz, $CDCl_3$) 50.3 (CH_2N), 53.4 (CH_3), 55.9 (CH_2Ph), 67.6 (CH_2O), 97.7 (C-5″), 99.3 (C-7), 100.8 (C-2‴/6‴), 106.4 (C-5), 113.4 (C-4‴), 114.8 (C-m), 119.4 (C-3a), 127.8 (C-6), 129.2 (C-i), 129.3 (C-o), 131.2 (C-3), 131.9 (C-4), 141.2 (C-7a), 141.6 (C-1‴), 153.4 (C-3‴/5‴), 157.5 (C-6″), 159.8 (C-p), 160.3 (C-2″), 161.6 (C-4″); MS (ESI$^+$): *m*/*z* 593 (100, M^++H); elemental analysis calcd for $C_{33}H_{36}N_8O_3$ (592.69): C, 66.87, H, 6.12, N, 18.91; found: C, 66.51, H, 6.14, N, 18.99.

4.2.11. N^2-(3,5-Dimorpholinophenyl)-N^4-(1-(4-methoxybenzyl)-1*H*-indazol-4-yl)-N^4-methylpyrimidin-2,4-diamine (11)

Compound **8a** (137 mg, 0.36 mmol) and compound **9** (104 mg, 0.40 mmol) were dissolved in anhydrous dioxane (5 mL) and *p*-TsOH in catalytic amounts was added. Afterwards, the reaction mixture was stirred at 100 °C for 1 d. The reaction was quenched with water (15 mL) and half-saturated bicarbonate solution (4 mL). The aqueous layer was extracted with EtOAc (3 × 15 mL). The combined organic layers were dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification was done by column chromatography ($CHCl_3$–MeOH = 98:2) to yield compound **11** (239 mg, 78%) as light red solid. Mp 111 °C; R_f = 0.25 (EtOAc); δ_H (400 MHz, $CDCl_3$) 3.16 (8H, t, ^3J = 4.7, CH_2N), 3.61 (3H, s, CH_3), 3.77 (3H, s, CH_3), 3.84 (8H, t, ^3J = 4.7, CH_2O), 5.55 (2H, s, CH_2), 5.70 (1H, d, ^3J = 6.0, 5″-H), 6.17 (1H, s, 4‴-H), 6.85 (2H, dd, ^3J = 8.7, H-m), 6.90 (2H, d, ^4J = 2.0, 2‴/6‴-H), 7.03 (1H, dd, ^3J = 6.7, ^3J = 7.8, 6-H), 7.17 (1H, s, NH), 7.22 (2H, dd, ^3J = 8.7, H-o), 7.33–7.36 (2H, m, 5/6-H), 7.80 (1H, d, ^3J = 6.0, 6″-H), 7.84 (1H, s, 3-H); δ_C (101 MHz, $CDCl_3$) 38.2 (CH_3), 50.0 (CH_2N), 53.1 (CH_3), 55.4 (CH_2Ph), 67.2 (CH_2O), 97.6 (C-5″), 98.6 (C-7), 99.8 (C-2‴/6‴), 108.7 (C-5), 114.3 (C-3′/5′), 119.0 (C-3a), 121.7 (C-4‴), 127.3 (C-6),128.7 (C-1′), 129.0 (C-2′/6′), 131.6 (C-3), 137.6 (C-4), 141.1 (C-7a), 141.9 (C-1‴), 153.0 (C-3‴/5‴), 156.0 (C-6″), 159.5 (C-4′), 159.9 (C-2″), 162.9 (C-4″); MS (ESI$^+$): *m*/*z* 607 (90, M^++H); elemental analysis calcd for $C_{34}H_{38}N_8O_3$ (606.72): C, 67.31, H, 6.31, N, 18.47; found: C, 67.24, H, 6.36, N, 18.23.

4.2.12. N^2-(3,5-Dimorpholinophenyl)-N^4-(1-(1-ethoxyethyl)-1*H*-indazol-4-yl)pyrimidin-2,4-diamine (12)

Compound **7b** (220 mg, 0.69 mmol) and compound **9** (182 mg, 0.69 mmol) were dissolved in anhydrous dioxane (5 mL), Cs_2CO_3 (615 mg, 1.89 mmol) was added. Xantphos and $Pd_2(dba)_3$ were added in catalytic amounts and the mixture was stirred at 100 °C for 12 h. The reaction was quenched with water (15 mL) and half-saturated bicarbonate solution (4 mL) was added. The aqueous layer was extracted with EtOAc (3 × 15 mL). The combined organic layers were dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification was done by column chromatography ($CHCl_3$–MeOH = 98:2) to yield compound **12** (276 mg, 73%) as pale brown solid. Mp 110 °C; R_f = 0.49 ($CHCl_3$–MeOH = 9:1); δ_H (400 MHz, $CDCl_3$) 1.14 (3H, t, ^3J = 7.1, CH_3CH_2), 1.80 (3H, d, ^3J = 6.1, CH_3CH), 3.13 (8H, t, ^3J = 4.8, CH_2N), 3.27 (1H, dq, ^2J = 9.3, ^3J = 7.1, CH_2), 3.46 (1H, dq, ^2J = 9.3, ^3J = 7.1, CH_2), 3.82 (8H, t, ^3J = 4.8, CH_2O), 5.89 (1H, q, ^3J = 6.1, CH), 6.16 (1H, t, ^4J = 2.0, 4″-4), 6.25 (1H, d, ^3J = 5.9, 5″-H) 6.78 (1H, d, ^4J = 2.0, 2″/6″-H), 7.06 (1H, s, NH), 7.17 (1H, s, NH), 7.33–7.38 (2H, m, 5/6-H), 7.48–7.50 (1H, m, H-7), 7.97 (1H, s, 3-H), 8.08 (1H, d, ^3J = 5.9, 5″-H); δ_C (101 MHz, $CDCl_3$) 15.1, 21.1 (2× CH_3), 49.9 (CH_2N), 64.0 (CH_2), 67.2 (CH_2O), 87.2 (CH), 97.4 (C-5′), 98.8 (C-7), 100.4 (C-2″/5″), 107.1 (C-5), 113.5 (C-4″), 119.7 (C-3a), 127.4 (C-6), 131.0 (C-3), 131.7 (C-4), 139.9 (C-7a), 141.3 (C-1″), 153.0 (C-3″/5″), 157.6 (C-6′), 160.3 (C-2′), 161.3 (C-4′); MS (ESI$^+$): *m*/*z* 545 (100, M^++H); elemental analysis calcd for $C_{29}H_{36}N_8O_3$ (544.65): C, 63.95, H, 6.66, N, 20.57; found: C, 64.01, H, 6.65, N, 20.55.

4.2.13. N^2-(3,5-Dimorpholinophenyl)-N^4-(1-(1-ethoxyethyl)-1*H*-indazol-4-yl)-N^4-methylpyrimidin-2,4-diamine (13)

Compound **8b** (133 mg, 0.40 mmol) and compound **9** (106 mg, 0.40 mmol) were dissolved in anhydrous dioxane (5 mL), Cs_2CO_3 (392 mg, 1.20 mmol) was added. Xantphos and $Pd_2(dba)_3$ were added in catalytic amounts and the mixture was stirred at 90 °C for 24 h. The reaction was quenched with water (15 mL) and half-saturated bicarbonate solution (4 mL) was added. The aqueous layer was extracted with EtOAc (3 × 15 mL). The combined organic layers were dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification was done by column chromatography ($CHCl_3$–MeOH = 98:2 → 95:5) to yield compound **13** (140 mg, 62%) as brown solid. Mp 82 °C; R_f = 0.57 ($CHCl_3$–MeOH = 9:1); δ_H (400 MHz, $CDCl_3$) 1.17 (3H, t, ^3J = 7.1, CH_3CH_2), 1.81 (3H, d, ^3J = 6.1, CH_3CH), 3.16 (8H, t, ^3J = 4.7, CH_2N), 3.31 (1H, dq, ^2J = 9.3, ^3J = 7.1, CH_2), 3.49 (1H, dq, ^2J = 9.3, ^3J = 7.1, CH_2), 3.62 (3H, s, CH_3), 3.84 (8H, t, ^3J = 4.7, CH_2O), 5.71 (1H, d, ^3J = 6.0, 5′-H), 5.91 (1H, q, ^3J = 6.1, CH), 6.17 (1H, s, ^4J = 1.8, 4″-H), 6.90 (2H, d, ^4J = 1.8, 2″/6″-H), 7.07 (1H, d, ^3J = 7.4, 5-H), 7.29 (1H, s, NH), 7.41 (1H, dd, ^3J = 7.4, ^3J = 8.4, 6-H), 7.68 (1H, d, ^3J = 8.4, 7-H), 7.81 (1H, s, 3-H), 7.82 (1H, d, ^3J = 6.0, 6′-H); δ_C (101 MHz, $CDCl_3$) 15.0, 21.1, 38.2 (3× CH_3), 50.0 (CH_2N), 64.0 (CH_2), 67.1 (CH_2O), 87.4 (CH), 97.5 (C-5′), 98.6 (C-7), 99.8 (C-2″/5″), 109.9 (C-5), 119.3 (C-3a), 122.3 (C-4″), 127.3 (C-6), 131.6 (C-3), 137.6 (C-4), 140.1 (C-7a), 141.9 (C-1″), 152.9 (C-3″/5″), 155.8 (C-6′), 159.9 (C-2′), 162.9 (C-4′); MS (ESI$^+$): *m*/*z* 559 (100, M^++H); elemental analysis calcd for $C_{30}H_{38}N_8O_3$ (558.67): C, 64.50, H, 6.86, N, 20.06; found: C, 64.45, H, 6.92, N, 20.01.

4.2.14. N^2-(3,5-Dimorpholinophenyl)-N^4-(methyl-1*H*-indazol-4-yl)-pyrimidin-2,4-diamine (14)

Compound **7c** (71 mg, 0.27 mmol) and compound **9** (80 mg, 0.27 mmol) were dissolved in anhydrous toluene (5 mL) and Cs_2CO_3 (352 mg, 1.08 mmol) was added. Xantphos and $Pd_2(dba)_3$ were added in catalytic amounts and the mixture was stirred at

90 °C for 24 h. The reaction was quenched with water (15 mL) and the aqueous layer was extracted with EtOAc (3 × 15 mL). The combined organic layers were dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification was done by column chromatography (EtOAc → EtOAc–EtOH = 10:1) to yield compound **14** (109 mg, 83%) as light brown solid. Mp 113 °C; R_f = 0.35 (EtOAc–EtOH = 3:1); δ_H (400 MHz, $CDCl_3$) 3.11 (8H, t, ^3J = 4.8, CH_2N), 3.81 (8H, t, ^3J = 4.8, CH_2O), 4.08 (1H, s, CH_3), 6.16 (1H, t, ^4J = 2.0, 4″-H), 6.23 (1H, d, ^3J = 5.8, 5′-H), 6.77 (2H, d, ^4J = 2.0, 2″/6″-H), 7.05 (1H, s, NH), 7.11 (1H, s, NH) 7.19 (1H, 't', ^3J = 4.8, 6-H), 7.34–7.38 (2H, m, 5/7-H), 7.94 (1H, s, 3-H), 8.07 (1H, d, ^3J = 5.8, 6′-H); δ_C (101 MHz, $CDCl_3$) 35.9 (CH_3), 49.8 (CH_2N), 67.1 (CH_2O), 97.5 (C-2″/6″), 98.8 (C-5′), 100.4 (C-7), 112.9 (C-5), 118.7 (C-3a), 127.3 (C-6), 130.4 (C-3), 131.6 (C-4), 141.3 (C-7a), 152.9 (C-5″/3″), 157.5 (C-1″), 160.2 (C-6′), 161.3 (C-2′), 171.1 (C-4′); MS (ESI^+): *m*/*z* 487 (100, M^++H); elemental analysis calcd for $C_{26}H_{30}N_8O_2$ (486.57): C, 64.18, H, 6.21, N, 23.03; found: C, 64.28, H, 6.13, N, 23.16.

4.2.15. N^2-(3,5-Dimorpholinophenyl)-N^4-methyl-N^4-(1-methyl-1*H*-indazol-4-yl)pyrimidin-2,4-diamine (15)

Compound **8c** (92 mg, 0.34 mmol) and compound **9** (89 mg, 0.34 mmol) were dissolved in anhydrous dioxane (5 mL) and Cs_2CO_3 (328 mg, 1.01 mmol) was added. Xantphos and $Pd_2(dba)_3$ were added in catalytic amounts and the mixture was stirred at 90 °C for 24 h. The reaction was quenched with water (15 mL) and the aqueous layer was extracted with EtOAc (3 × 15 mL). The combined organic layers were dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification was done by column chromatography ($CHCl_3$–MeOH = 98:2) to yield compound **15** (135 mg, 80%) as brown solid. Mp 78 °C; R_f = 0.69 (acetone–EtOAc = 2:1); δ_H (400 MHz, $CDCl_3$) 3.16 (8H, t, ^3J = 4.8, CH_2N), 3.61 (3H, s, CH_3), 3.83 (8H, t, ^3J = 4.8, CH_2O), 4.11 (3H, s, CH_3), 5.69 (1H, d, ^3J = 6.0, 5′-H), 6.13 (1H, t, ^4J = 2.0, 4″-H), 6.90 (2H, d, ^4J = 2.0, 2″/6″-H), 7.05 (1H, d, ^3J = 7.1, 5-H), 7.36–7.45 (2H, m, 6/7-H), 7.80 (1H, s, 3-H), 7.80 (1H, d, ^3J = 6.0, 6′-H); δ_C (101 MHz, $CDCl_3$) 6.0, 38.2 (2× CH_3), 50.0 (CH_2N), 67.1 (CH_2O), 97.4 (C-5′), 98.6 (C-7), 99.8 (C-2″/6″), 108.3 (C-5), 118.8 (C-4″), 121.2 (C-3a), 127.2 (C-6), 131.1 (C-3), 137.5 (C-4), 141.6 (C-7a), 141.9 (C-1″), 152.9 (C-3″/5″), 155.7 (C-6′), 159.9 (C-2′), 162.9 (C-4′); MS (ESI^+): *m*/*z* 501 (100, M^++H); elemental analysis calcd for $C_{27}H_{32}N_8O_2$ (500.60): C, 64.78, H, 6.44, N, 22.38; found: C, 64.91, H, 6.38, N, 22.50.

4.2.16. *N*-(2-Chloropyrimidin-4-yl)-1-(1-ethoxyethyl)-*N*-(3-fluoropropyl)-1*H*-indazol-4-amine (18a)

Compound **7b** (400 mg, 1.26 mmol) was dissolved in anhydrous DMF (5 mL), NaH (60% in mineral oil, 127 mg, 3.18 mmol) was added and the mixture was maintained at rt for 30 min. Afterwards, 1-fluoro-3-iodopropane (497 mg, 2.64 mmol) was added and the mixture was stirred at 80 °C overnight. After cooling to rt, water (15 mL) was added and the aqueous layer was extracted with EtOAc (3 × 15 mL). The combined organic layers were dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification was done by column chromatography (PE–EtOAc = 1:1) to yield compound **18a** (468 mg, 98%) as light red oil. R_f = 0.54 (PE–EtOAc = 1:2); δ_H (400 MHz, $CDCl_3$) 1.18 (3H, t, ^3J = 7.1, CH_3), 1.80 (3H, d, ^3J = 6.1, CH_3), 2.04–2.19 (2H, m, CH_2CH_2F), 3.31 (1H, dq, ^2J = 9.3, ^3J = 7.1, CH_2), 3.49 (1H, dq, ^2J = 9.3, ^3J = 7.1, CH_2), 4.20 (2H, br s, CH_2N), 4.54 (2H, dt, $^2J_{H,F}$ = 47.1, ^3J = 5.8, CH_2F), 5.88–5.96 (2H, m, CH, H_{Pyr}), 7.05 (1H, d, ^3J = 7.1, 5-H), 7.45 (1H, t, ^3J = 7.9, 6-H), 7.74 (1H, s, 3-H), 7.76 (1H, d, ^3J = 8.5, 7-H), 7.85 (1H, d, ^3J = 6.1, H_{Pyr}); δ_C (101 MHz, $CDCl_3$) 15.0, 21.1 (2× CH_3), 29.1 (d, $^2J_{C,F}$ = 19.8 Hz, CH_2CH_2F), 47.1 (d, $^3J_{C,F}$ = 5.4 Hz, CH_2N), 64.2 (CH_2O), 82.0 (d, $^1J_{C,F}$ = 165.7 Hz, CH_2F), 87.7 (CH), 103.9 (C-7), 111.2 (C-5′), 120.4 (C-3a), 122.4 (C-5), 127.5 (C-6), 130.9 (C-3), 134.8 (C-4), 140.2 (C-7a), 156.7 (C-2′), 160.8 (C-6′), 163.4 (C-4′); δ_F (376 MHz, $CDCl_3$) −220.7; MS (ESI+): *m*/*z* 380 (34, M^++H, ^{37}Cl), 378 (100, M^++H, ^{35}Cl); elemental analysis calcd for $C_{18}H_{21}ClFN_5O$ (377.84): C, 57.22, H, 5.60, N, 18.54; found: C, 57.25, H, 5.74, N 18.28.

4.2.17. *N*-(2-Chloropyrimidin-4-yl)-1-(1-ethoxyethyl)-*N*-(3-hydroxypropyl)-1*H*-indazol-4-amine (18b)

3-Bromopropanol (262 mg, 1.89 mmol) and NaI (300 mg, 2.00 mmol) were dissolved in acetone (5 mL) and the mixture was stirred at rt overnight. Afterwards, the solvent was changed against anhydrous DMF (5 mL), **7b** (364 mg, 1.15 mmol) and Cs_2CO_3 (1.2 g, 3.68 mmol) were added and the resulting mixture was stirred at 80 °C overnight. Afterwards, water was added, the mixture was extracted with EtOAc (3 × 20 mL), the combined organic layers were dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification was done by column chromatography (PE–EtOAc = 1:1 → 1:2) to yield compound **18b** (350 mg, 51%) as yellowish oil. R_f = 0.63 ($CHCl_3$–EtOH = 95:5); δ_H (400 MHz, $CDCl_3$) 1.17 (3H, t, ^3J = 7.0, CH_3), 1.70–1.83 (5H, m, CH_3/CH_2CH_2O), 3.31 (1H, dq, ^2J = 9.2, ^3J = 7.2, CH_2), 3.49 (1H, dq, ^2J = 9.2, ^3J = 7.2, CH_2), 3.72 (2H, br s, CH_2N), 3.89 (1H, br s, OH), 4.06–4.37 (2H, m, CH_2O), 5.88–5.95 (2H, m, CH, H_{Pyr}), 7.02 (1H, d, ^3J = 7.4, 5-H), 7.45 (1H, dt, ^3J = 7.4, ^3J = 8.5, 6-H), 7.74 (1H, s, 3-H), 7.78 (1H, d, ^3J = 8.5, 7-H), 7.85 (1H, d, ^3J = 6.0, H_{Pyr}); δ_C (101 MHz, $CDCl_3$) 15.0, 21.1, (2× CH_3), 31.0 (CH_2CH_2O), 46.6 (CH_2N), 58.4 (CH_2O), 64.2 (CH_2), 87.8 (CH), 103.9 (C-7), 111.4 (C-5′), 120.5 (C-5), 122.3 (C-3a), 127.6 (C-6), 130.8 (C-3), 134.2 (C-4), 140.2 (C-7a), 156.9 (C-6′), 160.6 (C-2′), 163.9 (C-4′); MS (ESI^+): *m*/*z* 398 (100, M^++Na, ^{35}Cl), 285 (42, 285 M^+−EOE–OH); elemental analysis calcd for $C_{18}H_{22}ClN_5O_2$ (375.85): C, 57.52, H, 5.90, N, 18.63; found: C, 57.55, H, 5.81, N, 18.74.

4.2.18. N^2-(3,5-Dimorpholinophenyl)-N^4-(1-(1-ethoxyethyl)-1*H*-indazol-4-yl)-N^4-(3-fluoropropyl)pyrimidin-2,4-diamine (19a) and N^2-(3,5-dimorpholinophenyl)-N^4-(3-fluoropropyl)-N^4-(1*H*-indazol-4-yl)pyrimidin-2,4-diamine (20)

Compounds **18a** (327 mg, 0.87 mmol) and **9** (207 mg, 0.79 mmol) were dissolved in anhydrous dioxane (10 mL), *p*-TsOH was added in catalytic amounts and the mixture was stirred at 90 °C for 48 h. After cooling to rt, saturated bicarbonate solution (15 mL) was added and the aqueous layer was extracted with EtOAc (3 × 15 mL). The combined organic layers were dried over $MgSO_4$ and the solvent was removed under reduced pressure to yield the crude compound **19a**. R_f = 0.48 ($CHCl_3$–EtOH = 95:5); δ_H (400 MHz, $CDCl_3$) 1.17 (3H, t, ^3J = 7.3, CH_3), 1.81 (3H, d, ^3J = 6.0, CH_3), 2.00–2.15 (2H, m, CH_2), 3.18 (8H, t, ^3J = 4.5, CH_2N), 3.32 (1H, dq, ^2J = 9.2, ^3J = 7.0, CH_2), 3.49 (1H, dq, ^2J = 9.2, ^3J = 7.0, CH_2), 3.86 (8H, t, ^3J = 4.7, CH_2O), 4.23 (2H, t, ^3J = 7.2, CH_2N), 4.50 (2H, dt, $^2J_{H,F}$ = 47.2, ^3J = 5.8, CH_2F), 5.56 (1H, d, ^3J = 6.0, H_{Pyr}), 5.92 (1H, q, ^3J = 6.0, CH), 6.17 (1H, t, ^3J = 1.6, H_{Ar}), 6.83 (2H, d, ^3J = 1.6, H_{Ar}), 7.06 (1H, d, ^3J = 7.5, 5-H), 7.43 (1H, dd, ^3J = 6.9, ^3J = 8.2, 6-H), 7.71 (1H, ^3J = 8.2, 7-H), 7.77 (1H, d, ^4J = 0.8 Hz, 3-H), 7.80(1H, ^3J = 6.0 Hz, H_{Pyr}); δ_F (376 MHz, $CDCl_3$) −225.2; MS (ESI+): *m*/*z* 627 (72, M^++Na); 605 (100, M^++H); 432 (76, M^+−2 × Morph). Subsequently, the crude compound **19a** (approx. 300 mg) was treated with 1 M HCl (6 mL) for 1.5 h at rt. Afterwards, saturated bicarbonate solution was added, the mixture was extracted with EtOAc (4 × 15 mL), the combined organic layers were dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification was done by column chromatography (DCM–MeOH = 19:1) to yield compound **20** (381 mg, 27% after two steps) as yellowish solid. Mp 196 °C; R_f = 0.30 (DCM–EtOH = 19:1); δ_H (400 MHz, $CDCl_3$) 1.98–2.12 (2H, m, CH_2), 3.17 (8H, t, ^3J = 5.0, CH_2N), 3.85 (8H, t, ^3J = 4.6, CH_2O), 4.25 (2H, t, ^3J = 7.2, CH_2N), 4.50 (2H, dt, $^2J_{H,F}$ = 47.2, ^3J = 5.7, CH_2F), 5.56 (1H, d, ^3J = 6.1, H_{Pyr}),

6.18 (1H, t, ^3J = 1.9, H_{Ar}), 6.83 (2H, d, ^3J = 1.9, H_{Ar}), 7.07 (1H, d, ^3J = 6.9, 5-H), 7.22 (1H, br s, NH), 7.43–7.53 (2H, m,6-H, 7-H), 7.78 (1H, d, ^3J = 6.1, H_{Pyr}), 7.87 (1H, s, 3-H); δ_C (101 MHz, $CDCl_3$) 29.5 (d, $^2J_{C,F}$ = 19.3 Hz, CH_2CH_2F), 46.5 (d, $^3J_{C,F}$ = 5.2 Hz, CH_2N), 50.0 (CH_2N), 67.1 (CH_2O), 82.0 (d, $^1J_{C,F}$ = 165.4 Hz, CH_2F), 97.8, 98.9, 100.5, 109.6, 120.5, 121.4, 127.9, 133.1, 135.7, 141.4, 141.8, 153.0, 155.2, 159.5, 162.8; δ_F (376 MHz, $CDCl_3$) −220.0; MS (ESI+): *m*/*z* 533 (100, M^++H); elemental analysis calcd for $C_{28}H_{33}FN_8O_2$ (532.61): C, 63.14, H, 6.25, N, 21.04; found: C, 63.25, H, 6.21, N, 20.99.

4.2.19. 3-((2-(3,5-Dimorpholinophenylamino)pyrimidin-4-yl)-(1-(1-ethoxyethyl)-1*H*-indazol-4-yl)amino)propan-1-ol (19b)

Compounds **9** (309 mg, 0.82 mmol) and **18b** (201 mg, 0.69 mmol) were dissolved in anhydrous dioxane (6 mL), *p*-TsOH was added in catalytic amounts and the mixture was stirred at 90 °C overnight. After cooling to rt, saturated bicarbonate solution (15 mL) was added and the aqueous layer was extracted with EtOAc (3 × 15 mL). The combined organic layers were dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification was done by column chromatography (PE–EtOAc = 2:1 → 1:1) to yield compound **19b** (320 mg, 77%) as yellowish oil. R_f = 0.52 (DCM–MeOH = 4:1); δ_H (400 MHz, $CDCl_3$) 1.17 (3H, t, ^3J = 7.0 Hz, CH_3), 1.69–1.77 (2H, m, CH_2), 1.81 (3H, d, ^3J = 6.1, CH_3), 3.16 (8H, t, ^3J = 4.8, CH_2N), 3.31 (1H, dq, ^2J = 9.2, ^3J = 7.0, CH_2), 3.49 (1H, dq, ^2J = 9.2, ^3J = 7.0, CH_2), 3.66 (2H, t, ^3J = 5.5, CH_2N), 3.84 (8H, t, ^3J = 4.8, CH_2O), 4.25 (2H, br s, CH_2OH), 5.51 (1H, d, ^3J = 5.9, 5′-H), 5.91 (1H, q, ^3J = 6.1, CH), 6.20 (1H, t, ^3J = 1.7, H_{Ar}), 6.77 (2H, d, ^3J = 1.5, H_{Ar}), 7.03 (1H, d, ^3J = 7.4, 5-H), 7.09 (1H, s, NH), 7.42 (1H, dd, ^3J = 7.4, ^3J = 8.4, 6-H), 7.70–7.81 (3H, m, 3-H/6′-H/7-H); δ_C (101 MHz, $CDCl_3$) 15.0, 21.1 (2× CH_3), 31.2 (CH_2), 46.0 (CH_2N), 50.0 (CH_2N), 58.6 (CH_2OH), 64.1 (OCH_2), 67.1 (OCH_2), 87.5 (CH), 97.6, 99.3, 101.1, 110.6, 120.9, 122.9, 127.4, 131.3, 135.3, 140.1, 141.1, 153.0, 156.2, 159.1, 163.2; MS (ESI+): *m*/*z* 603 (5, M^++H), 531 (100, M^+−EOE); elemental analysis calcd for $C_{32}H_{42}N_8O_4$ (602.73): C, 63.77, H, 7.02, N, 18.59; found: C, 63.85, H, 7.00, N, 18.63.

4.2.20. 1-(2-((3,5-Dimorpholinophenyl)amino)pyrimidin-4-yl)-1-(1-(1-ethoxyethyl)-1*H*-indazol-4-yl)azetidin-1-ium methanesulfonate (23)

Compound **19b** (538 mg, 0.89 mmol) was dissolved in DCM (20 mL), DIPEA (5 mL) and MsCl (307 mg, 2.68 mmol) were added at 0 °C. After stirring at 0 °C for 1 h, the reaction was quenched with saturated bicarbonate solution (15 mL), the aqueous layer extracted with DCM (3 × 10 mL), the combined organic layers dried over Na_2SO_4 and the solvent removed to obtain the crude **22**. Subsequently, the crude was dissolved in ACN (5 mL), AgOMs (40 mg, 0.20 mmol) was added and the mixture stirred in the dark at rt for 1 h. The solvent was changed to DCM (5 mL), the solid was filtered and washed with DCM (2 mL). Next, the solvent was removed and the crude **23** purified by column chromatography (DCM–MeOH = 100:1 → 10:1) to yield **23** (195 mg, 33%) as syrup. R_f = 0.20 (DCM–MeOH = 10:1); δ_H (400 MHz, $CDCl_3$) 1.15 (3H, t, ^3J = 7.0 Hz, CH_3), 1.78 (3H, d, ^3J = 6.0 Hz, CH_3), 2.48–2.58 (2H, m, Spiro-CH_2), 2.67 (3H, s, Ms), 3.12 (8H, t, ^3J = 4.9 Hz, CH_2N), 3.29 (1H, dq, ^2J = 9.3 Hz, ^3J = 7.0 Hz, CH_2), 3.48 (1H, dq, ^2J = 9.3 Hz, ^3J = 7.0 Hz, CH_2), 3.77 (8H, t, ^3J = 4.9 Hz, CH_2O), 3.92 (2H, m, Spiro-NCH_2), 4.75 (2H, t, ^3J = 5.8 Hz, Spiro-CH_2), 5.66 (1H, d, ^3J = 6.0 Hz, H_{pyr}), 5.92 (1H, q, ^3J = 6.0 Hz, CH), 6.26 (1H, s, H_{Ar}), 6.76 (2H, s, H_{Ar}), 7.18 (^3J = 7.4 Hz, 1H, d, H-5), 7.48 (^3J = 6.1 Hz, ^3J = 7.4 Hz, 1H, t, H-6), 7.77 (^3J = 6.0 Hz, 1H, d, H_{Pyr}), 7.85 (^3J = 6.1 Hz, 1H, d, H-7), 7.94 (1H, s, H-3), 10.3 (1H, s, NH); δ_C (101 MHz, $CDCl_3$) 14.9 (CH_3), 20.2 (CH_2), 21.1 (CH_3), 39.6 (Ms), 46.2, 49.2, 49.5, 64.3 (4× CH_2N), 67.0 (CH_2O), 87.9 (CH), 96.9 (C-2″/-6″), 101.4 (C-4″), 104.6 (C-7), 112.8 (C-5′), 120.8 (C-3a), 120.9 (C-5), 127.6 (C-6), 130.1 (C-3), 133.3 (C-4), 140.1 (C-7a), 140.3 (C-1″), 152.6 (C-3″/-5″),153.3 (C-6′), 154.7 (C-2′), 157.0 (C-4′); MS (ESI+): *m*/*z* 585 (90, M^+−OMs), 513 (100, M^+−OMs−EOE); MS (ESI−): *m*/*z* 95 (100, OMs^-); elemental analysis calcd for $C_{33}H_{44}N_8O_6S$ (680.82): C, 58.22, H, 6.51, N, 16.46; found: C, 58.19, H, 6.45, N, 16.38.

4.2.21. 1-(2-((3,5-Dimorpholinophenyl)amino)pyrimidin-4-yl)-1-(1-(1-ethoxyethyl)-1*H*-indazol-4-yl)azetidin-1-ium fluoride (24)

Compound **19b** (538 mg, 0.89 mmol) was dissolved in DCM (20 mL), DIPEA (5 mL) and MsCl (307 mg, 2.68 mmol) were added at 0 °C. After stirring at 0 °C for 30 min, the reaction was quenched with saturated bicarbonate solution (15 mL), the aqueous layer extracted with DCM (3 × 10 mL), the combined organic layers dried over Na_2SO_4 and the solvent removed to obtain the crude **22**. Subsequently, the crude was dissolved in ACN (5 mL), AgF (40 mg, 0.20 mmol) was added and the mixture was stirred in the dark at rt for 1 h. The solvent was changed by DCM (5 mL), the solid filtered and washed with DCM (2 mL). Next, the solvent was removed and the crude product was purified by column chromatography to yield **24** (146 mg, 27%) as syrup/solid. R_f = 0.20 (DCM–MeOH = 10:1); δ_H (400 MHz, $CDCl_3$) 1.17 (3H, t, ^3J = 7.0 Hz, CH_3), 1.80 (3H, d, ^3J = 6.1 Hz, CH_3), 2.39–2.52 (2H, m, Spiro-CH_2), 3.11 (8H, t, ^3J = 4.6 Hz, CH_2N), 3.27–3.36 (1H, m, CH_2), 3.50 (1H, dq, ^2J = 9.3 Hz, ^3J = 7.1 Hz, CH_2), 3.77 (8H, t, ^3J = 4.6 Hz, CH_2O), 3.87 (2H, m, Spiro-NCH_2), 4.46 (2H, m, Spiro-CH_2), 5.17 (1H, br s, H_{Ar}), 5.93 (1H, q, ^3J = 6.1 Hz, CH), 6.14 (1H, br s, H_{pyr}), 6.38 (2H, br s, H_{Ar}), 7.14 (^3J = 7.4 Hz, 1H, d, H-5), 7.48 (^3J = 8.6 Hz, ^3J = 7.5 Hz, 1H, dd, H-6), 7.58 (1H, br s, H_{Pyr}), 7.83 (^3J = 8.6 Hz, 1H, d, H-7), 7.88 (1H, s, H-3); δ_C (101 MHz, $CDCl_3$) 14.9 (CH_3), 19.4 (CH_2), 21.1 (CH_3), 41.3, 42.5, 54.1, 64.2 (4× CH_2N), 66.9 (CH_2O), 87.6 (CH), 112.5, 119.8, 120.9, 127.7, 130.0, 133.5, 139.9, 153.0, 154.9, 157.6; δ_F (376 MHz, $CDCl_3$) −120.0; MS (ESI+): *m*/*z* 585 (73, M^+−F), 513 (100, M^+−F−EOE); elemental analysis calcd for $C_{32}H_{41}FN_8O_3$ (604.72): C, 63.56, H, 6.83, N, 18.53; found: C, 63.42, H, 6.89, N, 18.61.

4.3. Radiosynthesis

4.3.1. N^2-(3,5-Dimorpholinophenyl)-N^4-(1*H*-indazol-4-yl)-N^4-[^{11}C]-methylpyrimidin-2,4-diamine ([^{11}C]17)

Labeling precursor **12** (approx. 1.2 mg) dissolved in DMF (250 μL) and 0.5 M NaOH (40 μL) was placed into the reacting vessel of the synthesizer unit. [^{11}C]CH_3I was transferred in a stream of Helium into the vessel at −20 °C. After completion, the reactor was sealed and heated at 80 °C for 3 min. Then, the reactor was cooled to 60 °C and 1 M HCl (200 μL) was added and the mixture was maintained for 2 min at 60 °C. Afterwards, the mixture was transferred onto a semi-preparative HPLC column. The product eluting between 5 and 7 min was separated and diluted with 30 mL of water, passed over a PR-18 cartridge and eluted with 0.75 mL of EtOH and diluted with 6.5 mL of E153 solution. In a typical experiment approx. 200 MBq of compound **[^{11}C]17** could be obtained within 20 min after EOB starting from 1 to 1.5 GBq [^{11}C]CH_4 (30–35% d.c. yield based on [^{11}C]CH_4).

4.4. Molecular modeling

Docking was performed using Glide module.[40] Protein coordinates were extracted from the X-ray crystal structure of the EphB4 kinase domain in complex with a potent inhibitor (code 2x9f in PDB). A grid box of 20 Å × 20 Å × 20 Å was centered on the center of mass of the inhibitor in this crystal structure covering the ATP-binding site of the enzyme. The module LigPrep (version 2.5, Schrödinger, LLC, NY) was used to assign ionization states,

stereochemistries, and ring conformations of the sketched ligands. Docking parameters were used as in previous works,[41] in extraprecision (XP) mode during the search. The more energetically favorable conformation was selected as the best pose.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2015.06.040.

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