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Meridianin derivatives as potent Dyrk1A inhibitors and neuroprotective agents ☆

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ABSTRACT

Meridianins are a group of marine-derived indole alkaloids which are reported to possess kinase inhibitory activities. In the present Letter, we report synthesis of *N*1-substituted and *C*-ring modified meridianin derivatives and their evaluation as Dyrk1A inhibitors and neuroprotective agents. Among the library of 52 compounds screened, morpholinoyl linked derivative **26b** and 2-nitro-4-trifluoromethyl phenyl sulfonyl derivative **29v** displayed potent inhibition of Dyrk1A with IC_{50} values of 0.5 and 0.53 μM, respectively. The derivative **26b** also inhibited Dyrk2 and Dyrk3 with IC_{50} values of 1.4 and 2.2 μM, respectively showing 2.2 and 4.4 fold selectivity for Dyrk1A with respect to Dyrk2 and Dyrk3. The compound **26b** was not cytotoxic to human neuroblastoma SH-SY5Y cells (IC_{50} >100 μM) and it displayed significant neuroprotection against glutamate-induced neurotoxicity in these cells at 10 μM. Molecular modelling studies of compound **26b** led to identification of key interactions in the binding site of Dyrk1A and the possible reasons for observed Dyrk1A selectivity over Dyrk2.

Dual-specificity Tyrosine-regulated Kinases (Dyrks) belong to the CMGC family of eukaryotic protein kinase superfamily. This group of kinases are further divided into two classes: (a) class 1 kinases (Dyrk1A and 1B) that has N-terminal nuclear localization signal and a C-terminal PEST region and (b) class 2 kinases (Dyrk2, 3 and 4), which lacks these motifs and are predominantly cytosolic.[1] Dyrk1A gets over-expressed in several neurodegenerative diseases including Down syndrome and Alzheimer's disease; thus it has been considered as a molecular target for these diseases.[2–5] Considering the importance of Dyrk1A in Down syndrome genesis and its role in signal transduction, many efforts have been made to discover potent inhibitors of Dyrk1A. Harmine (**1**) is a β-carboline alkaloid possessing potent Dyrk1A inhibitory activity with IC_{50} value of 80 nM.[6–8] Another natural product inhibitor (IC_{50} 330 nM) of Dyrk1A is epigallocatechin gallate (**2**), which is a major catechin component of green tea.[9,10] INDY (**3**) and its analog **4** are benzothiazoles possessing Dyrk1A inhibitory activity, with IC_{50} values of 0.24 and 0.93 μM.[11] A marine natural product leucettine B derivative **5** possess potent Dyrk1A inhibition with IC_{50} of 0.04 μM.[12,13] Meridianin analog **6** displayed potent inhibition of Dyrk1A with IC_{50} of 0.034 μM.[14] A structurally similar azo-indole **7** is the most potent in vitro inhibitor of Dyrk1A reported till date.[15] Acridine derivative **8** displayed inhibition of Dyrk1A, Dyrk2 and Dyrk3 enzymes with IC_{50} values of 0.1, 0.002, 0.019 μM.[16] Schmitt et al.[17] discovered a substituted thiophene **9** possessing inhibition of Dyrk1A, Dyrk1B and Dyrk2 with IC_{50} values of 0.1, 0.07 and 0.04 μM, respectively. The chemical structures of compounds **1**–**9** are shown in Figure 1.

Meridianins[18,19] are indole alkaloids substituted at the C-3 position by 2-aminopyrimidine ring. The meridianins have been evaluated against number of kinases such as Cdk1, Cdk2, Cdk5, PKA, PKG, GSK-3β, CK1, KDR, IGF-1R, c-Met, RET, c-Src, c-Abl, HER-1, Erk2 and Dyrk1A.[14,20–22] Recently, Moreau and co-workers[14] evaluated meridianin analogs against 5 kinases (Cdk5/p25, CK1δ/ε, GSK-3α/β, Dyrk1A, and CLK1) and for their in vitro antiproliferative activities against human cancer cell lines (PC3, DU145, PA1, SH-SY5Y, IMR-32 and MCF7). In the present study, our aim was to further explore the SAR of meridianins for Dyrk1A inhibition and investigate their neuroprotective activity.

Thus, herein we prepared a series of *N*-substituted meridianin derivatives and screened for Dyrk1A inhibition. The selectivity of key compound over other Dyrk's (Dyrk2 and Dyrk3) was also studied. The molecular modeling studies were carried out to

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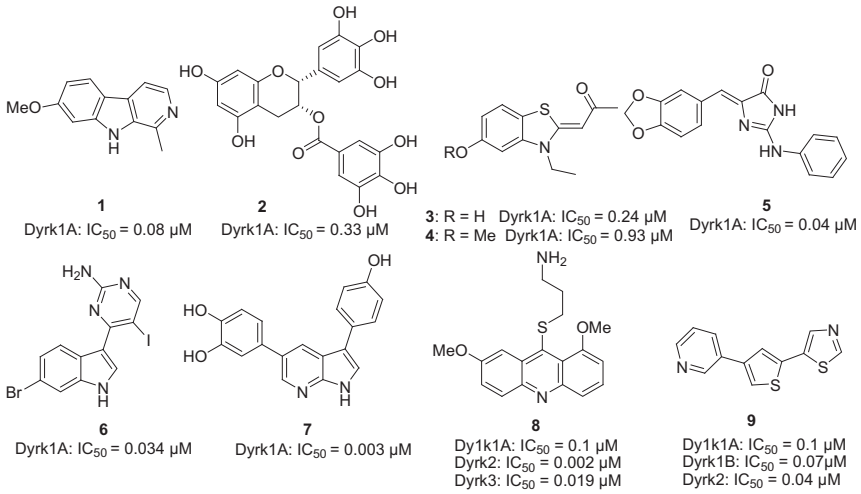
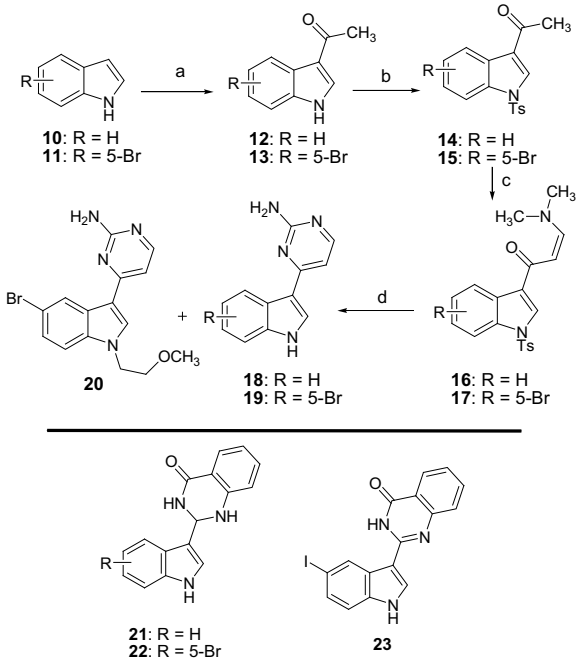


Figure 1. Chemical structures of Dyrk inhibitors.

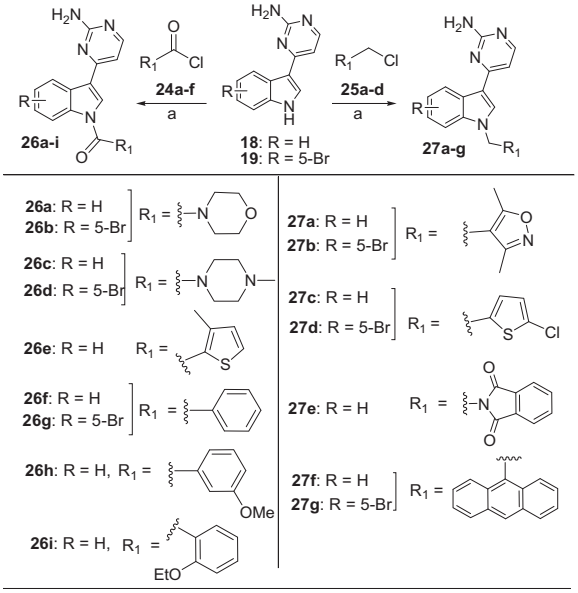
understand the possible reason for selective Dyrk1A inhibition over Dyrk2.

Meridianin C (**19**) and G (**18**) were synthesized in four steps starting from commercially available indoles **10**–**11** as shown in Scheme 1[23] The synthesized meridianins C and G were characterized by comparison of their spectral data with literature values.[18,19]

The *N*-acyl and *N*-alkyl derivatives **26a**–**i** and **27a**–**g** were prepared as described earlier[24] and is depicted in Scheme 2. Similarly, a library of *N*-sulfonyl derivatives **29a**–**ad** were prepared from meridianins C and G. Treatment of meridianins C and G with different substituted sulfonyl chlorides **28a**–**t** in presence of DIPEA and DMAP produced *N*-sulfonyl products **29a**–**ad** in 38–87% yield (Scheme 3).[25] The C-ring modified meridianin analogs **21**–**23** were



Scheme 1. Synthesis of meridianin G (**18**) and meridianin C (**19**). Reagents and conditions: (a) AcCl (2 equiv), $SnCl_4$ (2 equiv), toluene, 0 °C, 2 h, 84–95%; (b) TsCl (1.1 equiv), DIPEA (1.5 equiv), DMAP (0.05 equiv), DCM, rt, 20 h, 72–85%; (c) DMF-DMA (1.5 equiv), DMF, 110 °C, 4 h; 50–55%; (d) guanidine HCl (1.5 equiv), 2-methoxyethanol, K_2CO_3 (2 equiv), 120 °C, 24 h, 55–66%.

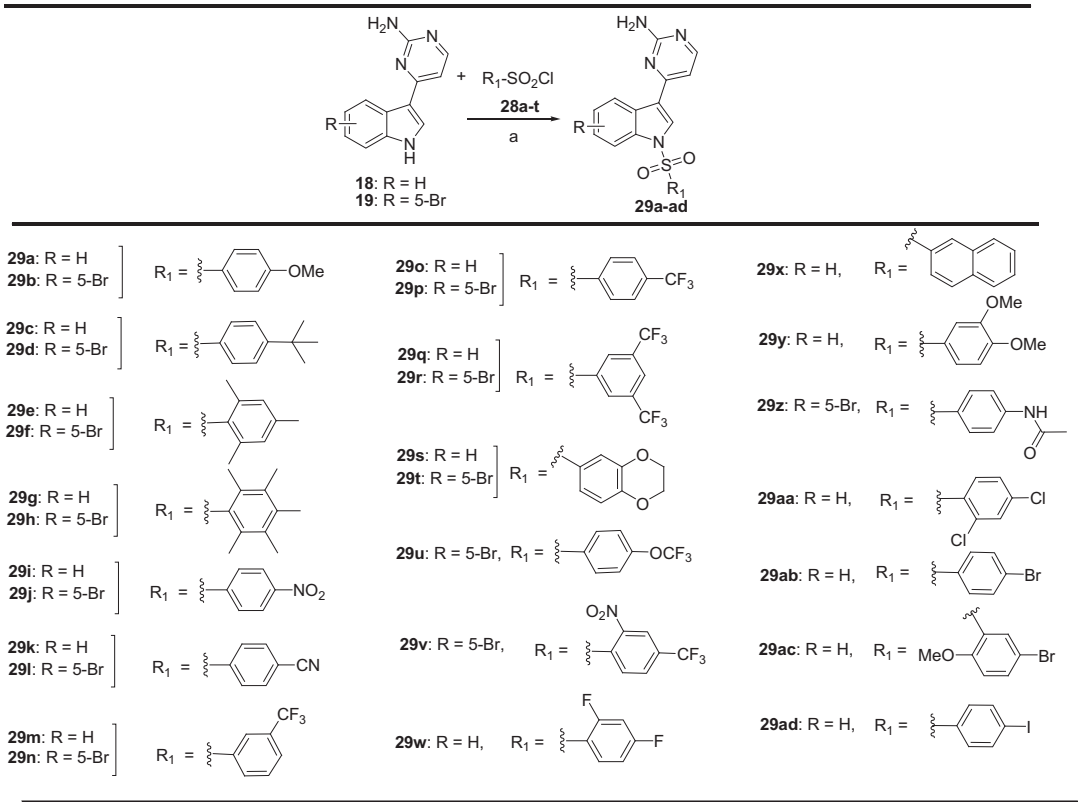


Scheme 2. Synthesis of *N*1-substituted meridianin derivatives **26a**–**i** and **27a**–**g**. Reagents and conditions: (a) K_2CO_3 (2 equiv), alkyl halide or acyl halide (1 equiv) ACN, 12 h, rt, 32–86%.

also prepared (structures shown in Scheme 1).[24] All synthesized derivatives were characterized by ^1H, ^{13}C NMR, IR, HRMS and melting point analysis.

All derivatives were tested for Dyrk1A inhibition at 0.5 μM and cytotoxicity in SH-SY5Y cells. Meridianin G (**18**) and C (**19**) along with several derivatives showed significant Dyrk1A inhibition activity. Based on the preliminary screening results, the IC_{50} was determined for 6 derivatives viz. **20**, **26b**, **26f**, **26g**, **27b** and **29v**. The *N*-morpholinoyl derivative **26b** showed potent inhibition of Dyrk1A with IC_{50} value of 0.5 μM. *N*-Benzoyl derivatives **26f** and **26g** showed Dyrk1A inhibition with IC_{50} values of 3.1, 0.835 μM, respectively. 2-Nitro-4-trifluoromethyl phenyl sulfonyl derivative of meridianin C **29v** showed IC_{50} value of 0.53 μM (Table 2).

All compounds were screened for cytotoxicity in SH-SY5Y cells at 30 μM, wherein most of the compounds were not cytotoxic (Table 1). The cytotoxicity IC_{50} value of three compounds **26b**, **26g** and **29v**, which exhibited better inhibition of Dyrk1A, was then determined. Compounds **26b** and **26g** were not cytotoxic up to



Scheme 3. Synthesis of *N*1-substituted meridianin derivatives **29a–ad**. Reagents and conditions: (a) R_1-SO_2Cl (1.1 equiv), DIPEA (1.5 equiv), DMAP (0.05 equiv), DCM, rt, 20 h, 38–87%.

Table 1
Dyrk1A inhibition and SH-SY5Y cell viability of meridianin derivatives

Entry	Dyrk1A (% inhibition)[a]	SH-SY5Y (% viability)[b]	Entry	Dyrk1A (% inhibition)[a]	SH-SY5Y% viability[b]
18	29.8	91.4	**29e**	15.7	40
19	56.7	92.9	**29f**	23.7	109
20	27.2	75.3	**29g**	7.6	49
21	2	83.1	**29h**	21.3	107
22	9	41.5	**29i**	4.9	56
23	20	24	**29j**	16.8	59
26a	1.4	97.3	**29k**	0	110
26b	56.7	87	**29l**	22.8	106
26c	0.4	82	**29m**	0	76.1
26d	22.6	91.8	**29n**	11	38.8
26e	24.6	82	**29o**	0	95.6
26f	35.3	106	**29p**	0	85
26g	40.7	65.1	**29q**	9.8	72.4
26h	12.2	94.6	**29r**	10.1	76.4
26i	2.3	46.6	**29s**	14.7	59.4
27a	13.2	88.5	**29t**	2.1	112
27b	33.1	83.1	**29u**	0	74
27c	20	115	**29v**	36.9	15.2
27d	8.5	24	**29w**	0	99.1
27e	0	101	**29x**	6.3	61.6
27f	9.6	96	**29y**	0	127
27g	14.2	79.8	**29z**	24.3	71.6
29a	10.6	58.8	**29aa**	13.2	55.4
29b	3.9	99	**29ab**	4.5	91.4
29c	12.9	86.3	**29ac**	4.5	96.2
29d	12.9	109	**29ad**	2.8	57.5

[a] Compounds were tested toward Dyrk1A at 0.5 μM.
[b] Compounds were tested for cytotoxicity in SH-SY5Y cell line at 30 μM.

100 μM (IC_{50} >100 μM), however the sulfonyl linked derivative **29v** displayed cytotoxicity with IC_{50} value of 14 μM.

Based on the cytotoxicity results, next we selected compounds **26b** and **26g** for neuroprotective activity in human neuroblastoma

Table 2
IC_{50} values of selected compounds for Dyrk1A inhibition

Entry	Dyrk1A (IC_{50}, μM)
19	0.33[a]
20	1.3
26b	0.5
26f	3.1
26g	0.83
27b	1.8
29v	0.53

[a] Literature value.[14]

Table 3
Neuroprotective effect of compounds **26b** and **26g** in human neuroblastoma SH-SY5Y cells

Compound	% viability (±SD) of SH-SY5Y cells
Control	100
Glutamate (250 μM)	73.8 ± 4.5
Glutamate (250 μM) + **26b** (50 μM)	86.4 ± 3.8
Glutamate (250 μM) + **26b** (10 μM)	82.5 ± 3.7
Glutamate (250 μM) + **26g** (50 μM)	94.1 ± 4.6
Glutamate (250 μM) + **26g** (10 μM)	93.2 ± 2.1

Table 4
Activity of compound **26b** against three Dyrk's

Dyrk enzyme	IC_{50}, μM	Fold selectivity for Dyrk1A
Dyrk1A	0.5	–
Dyrk2	1.4	2.8
Dyrk3	2.2	4.4

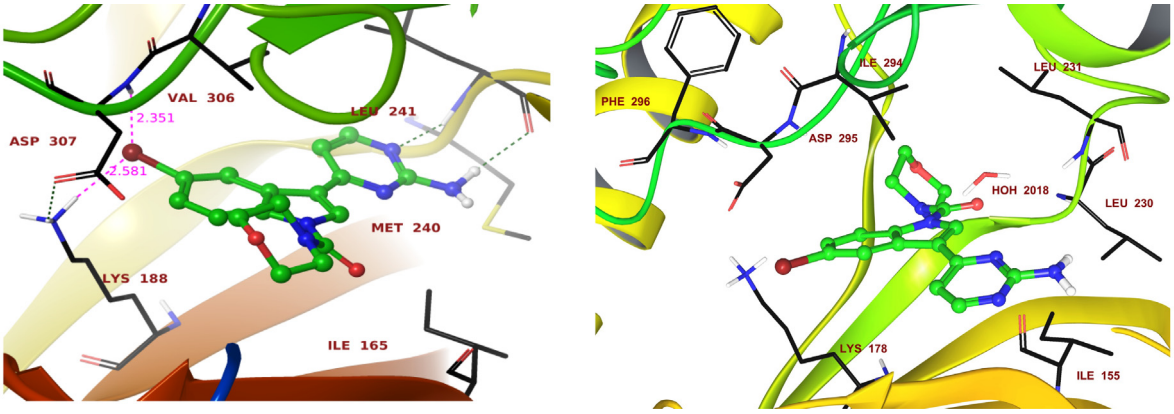


Figure 2. Interactions of meridianin derivative **26b** with Dyrk1A (a) and Dyrk2 (b).

Table 5
The comparison of binding site residues of Dyrk1A and Dyrk2[a]

Dyrk1A	Dyrk2
Ile165	Ille155
Lys188	Lys178
Met240	**Leu230**
Leu241	Leu231
Val306	**Ile294**
Asp307	Asp295
Phe308	Phe296
Gly309	Gly297

[a] The blue colored residues are key differences in these two enzymes, which might be responsible for observed selectivity of compound **26b** towards one enzyme than other.

SH-SY5Y cells against glutamate-induced neurotoxicity. The SH-SY5Y cells were differentiated for one week with 10 μM of retinoic acid. Differentiated cells were used for neuroprotective effect against glutamate induced toxicity. Both compounds at 10 as well as 50 μM, provided significant protection against the toxicity induced by glutamate. Results are shown in Table 3.

Compound **26b** was further screened against a panel of 15 kinases including PKB-α, PKB-β, PKC-α, GSK-3β, CDK-2, CDk-9, Aurora A, Aurora B, AMPK, CK1, IKKβ, Abl, IGF-1R, IR and VEGFR at 0.5 μM. It did not show significant inhibition of any of these kinases. It showed moderate inhibition (26%) of PKC-α at this test concentration.

The *N*-morpholinoyl derivative **26b** was then investigated for selectivity over Dyrk2 and Dyrk3. Compound **26b** also showed inhibition of these Dyrk's with IC_{50} values of 1.4 and 2.2 μM, respectively. It showed 2.8 and 4.4-fold selectivity towards Dyrk1A with respect to Dyrk2 and Dyrk3 (Table 4).

In order to determine the mode of interaction and rationale for selectivity of meridianin derivative **26b** towards Dyrk1A, the molecular modeling studies were carried out. Similar to parent natural alkaloid meridianin C,[14] derivative **26b** also showed interactions at the hinge region of Dyrk1A. The 2-aminopyrimidine core mimics the ATP structure (adenine) interactions with the ATP binding site of Dyrk's. Secondary interactions were observed with the bulkier bromo functionality of inhibitor **26b** with Asp307 and Lys188 side chain in terms of hydrophobic and ionic interactions. Further, Val306 and DFG motif residues 307–309 displayed hydrophobic interactions. These hydrophobic interactions could be a plausible explanation for varying degree of Dyrk1A enzyme inhibition for **26b** (Fig. 2).

Furthermore, around 3-fold selective inhibition of Dyrk1A over Dyrk2 (target known for the cancer)[26] of **26b** can be explained by two factors: (a) difference in the binding orientation of **26b** with Dyrk1A and Dyrk2 and (b) hydrophobic interactions of **26b** with the conserved residues of Dyrk1A (Table 5)

The less activity towards Dyrk2 in comparison to Dyrk1A might be due to the loss of interactions of 2-aminopyrimidine core with Leu230 and Leu231 residues of hinge region. Instead of that, the compound **26b** orients differently and carbonyl group interacts at ATP binding site. This change in orientation of **26b** is might be due to its interactions with the conserved Met240 and Val306 residues of Dyrk1A. The opposite orientation of compound **26b** is stabilized by its H-bonding interactions with the H_2O-2018.

In conclusion, herein we have identified *N*1-substituted meridianin derivative **26b** as promising inhibitor of Dyrk1A and a neuroprotective agent. Compound **26b** displayed 3- and 4-fold selectivity for Dyrk1A with respect to Dyrk2 and Dyrk3. The promising neuroprotective activity in neuronal cells against glutamate induced neurotoxicity further indicates promise of this identified lead for further development as an anti-Alzheimer agent.

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Supplementary data

Supplementary data (experimental procedures and spectral data scans) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.05.034.

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25. *Synthesis*. The procedure for synthesis of **18**–**23**, **26a**–**i**, and **27a**–**g** has been described earlier.[24] The synthesis of meridianin sulfonamides **29a**–**ad** is as follows: To the solution of meridianin G (**18**) or meridianin C (**19**) in dichloromethane (5 ml) was added DMAP (0.05 equiv), aryl sulfonyl chloride (1.1 equiv) and *N*,*N*-diisopropylethylamine (1.5 equiv). The mixture was stirred at room temperature for 20 h. Reaction was then quenched by the addition of 10% HCl. This reaction mixture was extracted with dichloromethane (50 ml × 3), and combined organic layer was evaporated on rotary evaporator. The crude products were purified by silica gel column chromatography (mesh 100–200) using dichloromethane–methanol (99:1 to 97:3) to get the titled products **29a**–**ad**. Spectral data of all compounds is provided in Supplementary information. Data for representative compounds is provided here: (3-(2-Aminopyrimidin-4-yl)-5-bromo-1*H*-indol-1-yl)(morpholino) methanone (**26b**): yield: 87%; yellow solid; mp 244–246 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.86 (s, 1H), 8.44 (d, 1H, J = 1.6 Hz), 8.21–8.19 (m, 1H), 7.65–7.63 (m, 1H), 7.46 (t, 1H, J = 6.8 Hz), 7.14–7.12 (m, 1H), 6.71 (s, 1H), 5.72 (d, 1H, J = 2.0 Hz), 3.77 (s, 4H), 3.70 (s, 4H); ^{13}C NMR (CD_3OD+$CDCl_3$, 125 MHz): δ 162.35, 157.09, 156.14, 135.45, 131.47, 127.96, 127.05, 124.93, 123.92, 114.25, 114.03, 110.89, 106.40, 70.48, 58.43; IR (CHCl3): ν_{max} 3306, 2955, 2925, 2858, 1739, 1688, 1578, 1544, 1491, 1449, 1419, 1401, 1362, 1342, 1273, 1248, 1082, 1015 cm^{-1}; HRMS: m/z 402.0555 calcd for $C_{17}H_{16}BrN_5O_2$+H$^+$ (402.0560). 4-(5-Bromo-1-(2-nitro-4-(trifluoromethyl)phenyl sulfonyl)-1*H*-indol-3-yl)pyrimidin-2-amine (**29v**). Yield: 47%; yellow solid; mp 129–131 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.59 (d, 1H, J = 2.0 Hz), 8.24 (d, 1H, J = 5.6 Hz), 8.15 (s, 1H), 8.11 (d, 1H, J = 8.4 Hz), 7.95–7.91 (m, 2H), 7.70 (d, 1H, J = 8.8 Hz), 7.48 (dd, 1H, J = 8.8, 20 Hz), 7.01 (d, 1H, J = 5.6 Hz), 5.68 (s, 2H); ^{19}F NMR ($CDCl_3$, 376 MHz): δ −63.50 (s, 3F); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 162.99, 160.23, 158.62, 148.03, 133.94, 131.20, 129.90, 129.53, 129.51,128.80 (d, $^2J_{CF}$ = 22.5 Hz), 126.20, 122.71, 120.39, 118.67, 114.48, 108.27; IR ($CHCl_3$): ν_{max} 3401, 2955, 2924, 2854, 1738, 1573, 1553, 1459, 1439, 1398, 1362, 1323, 1269, 1188, 1084, 1020 cm^{-1}; HRMS: m/z 543.9716 calcd for $C_{19}H_{11}^{81}BrF_3N_5O_4S$ + H$^+$ (543.9740).
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