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Discovery of [(3-bromo-7-cyano-2-naphthyl)(difluoro)-methyl]phosphonic acid, a potent and orally active small molecule PTP1B inhibitor

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Abstract—A series of quinoline/naphthalene-difluoromethylphosphonates were prepared and were found to be potent PTP1B inhibitors. Most of these compounds bearing polar functionalities or large lipophilic residues did not show appreciable oral bioavailability in rodents while small and less polar analogs displayed moderate to good oral bioavailability. The title compound was found to have the best overall potency and pharmacokinetic profile and was found to be efficacious in animal models of diabetes and cancer.

Type-2 diabetes and obesity are reaching epidemic proportions and thus are becoming the leading causes of health care burdens around the world. One of the hallmarks of these diseases is insulin resistance, an attenuated response of insulin to regulate glucose homeostasis. Protein tyrosine phosphatase 1B (PTP1B), a negative insulin regulator, has been shown to play a role in developing insulin resistance and obesity. Mice lacking the PTP1B gene are resistant to diet-induced obesity and insulin resistance.[1] These findings are further corroborated by treating mice with PTP1B antisense oligonucleotides.[2] In addition, recent studies have shown that PTP1B also plays a role in tumorigenesis.[3] As a result, PTP1B inhibitors represent attractive pharmaceutical agents for treating type-2 diabetes, obesity, and cancer. Several approaches to find suitable PTP1B inhibitors have been reviewed recently.[4] A major drawback of most of these inhibitors is the lack of sufficient cell permeability and oral bioavailability due to the presence of highly negative charged polar residues in these inhibitors.[5] Some non-phosphonate inhibitors were recently shown to have off-target activity which complicated efficacy data interpretation.[6] Using oral bioavailability in rodents as a key parameter, we optimized a series of difluoromethylphosphonate PTP1B inhibitors with either a naphthalene or a quinoline template and discovered [(3-bromo-7-cyano-2-naphthyl)-(difluoro)methyl]phosphonic acid (**3g**) as a potent PTP1B inhibitor with a good pharmacokinetic profile. We report herein the SAR that led to the discovery of **3g**, its synthesis, pharmacokinetic profile in preclinical species, and its in vivo efficacies in mouse models of diabetes and cancer.

Potent PTP1B inhibitors such as **1** (Fig. 1 and Table 1) containing the difluoromethylphosphonate moiety have

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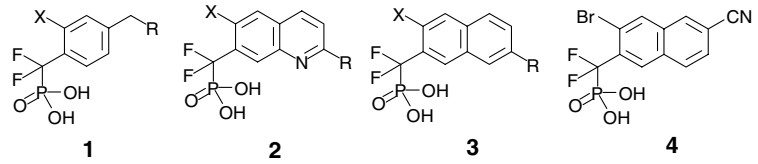


Figure 1. Structures of PTP1B inhibitors **1**, **2**, **3**, and **4**.

been reported previously.[7] Most of these compounds, however, exhibited poor cell permeability and poor pharmacokinetic profile.[5] Our current investigation started with the small quinoline difluoromethylphosphonate **2a** which did not display appreciable inhibitory activity ($IC_{50} > 50$ μM) against PTP1B in the fluorescein diphosphate (FDP) assay[8] (Table 1). Incorporation of an *ortho*-Br moiety gave **2b** with improved potency ($IC_{50} = 14$ μM). This observation was consistent with previous findings as shown in Table 1, **1b** versus **1c**.[7b] As a result, we focused our current effort on compounds bearing an *ortho*-Br moiety as shown in Table 1, **2c–i**.

Compound **2b** displayed good pharmacokinetic profile in rat with marginal oral bioavailability ($F = 5\%$) but good plasma drug exposure ($C_{max} = 43.3$ μM at 20 mg/kg) and long plasma elimination half life ($t_{1/2} > 8$ h). This compound, however, lacked the desired inhibitory activity against the PTP1B enzyme and thus was not a suitable tool for studying PTP1B inhibition in vivo. We then attempted to improve potency by exploiting the secondary binding site.[7a,9] From the high resolution (1.6 Å) X-ray structure of the PTP1B-**1c** complex (Fig. 2, only active site shown),[10] it was apparent that the secondary binding site, as characterized by the positively charged Arg24 and Arg254 residues, was not exploited. Docking the quinoline template into the active site suggested that it should be possible to extend to the secondary binding site by modifying the methyl group in **2b**. A variety of groups were modeled and the carboxylate in the 3-vinylbenzoate moiety (**2d**) was shown to have the potential to make excellent hydrogen binding interactions with Arg24 and Arg254 (Fig. 3). The styrene derivative **2c** was also prepared as a comparison.

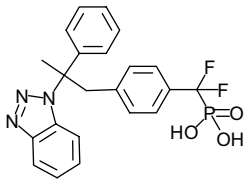
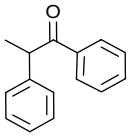
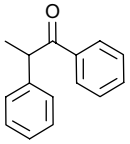
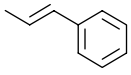
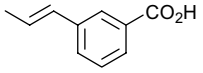
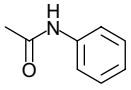
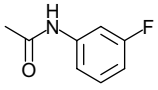
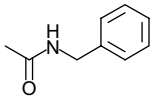
As illustrated in Table 1, incorporation of a styrene moiety gave **2c** with improved inhibitory activity over **2b** in the enzymatic assay ($IC_{50} = 160$ nM), suggesting that hydrophobic interactions were beneficial for activity. The incorporation of the carboxylate in **2d** improved PTP1B potency dramatically ($IC_{50} = 7$ nM), validating the prediction by molecular modeling. We further discovered that the double bond in **2c** could be replaced with an amide group, giving compounds **2e–g** with good intrinsic activities ($IC_{50} = 60$, 27 and 45 nM, respectively) without the requirement of a charged carboxylate residue. Unfortunately, all these compounds did not show any appreciable oral bioavailability in rodents. It was apparent that large lipophilic groups or polar residues were detrimental to oral bioavailability. The simple amide derivative *N*-methyl amide (**2h**) was significantly less active ($IC_{50} = 1.26$ μM) but the primary amide **2i** displayed good activity ($IC_{50} = 250$ nM) and appreciable oral absorption *albeit* with low plasma drug exposure ($C_{max} = 0.3$ μM at 10 mg/kg in C57BL/6 mice) compared to compound **2b**, again likely due to increased polarity.

The synthesis of quinoline derivatives **2b–i** is outlined in Scheme 1. 4-Bromo-3-iodoaniline (**5**)[11] was condensed with crotonaldehyde in the presence of chloranil and concentrated HCl in refluxing *i*-PrOH to give 6-bromo-7-iodo-2-methylquinoline (**6**) after separating from the region isomer by flash chromatography. The iodide was reacted with [(diethoxyphosphoryl)(difluoro)-methyl]zinc bromide using CuBr as the catalyst according to literature procedures[12] to yield compound **7**. Subsequent treatment of **7** with an excess of TMSBr[12] followed by quenching with either water or a base gave the corresponding phosphonate **2b**. Compound **7** was oxidized with SeO_2 to the corresponding aldehyde **8**. Reaction of **8** with Wittig reagents $ArCH_2P^+Ph_3Br^-$ followed by deprotection of the diethyl phosphonate moiety with TMSBr as described above gave phosphonates **2c–d**. Alternatively, **8** was oxidized further to the acid **9** by AgO in methanol. Reaction of acid **9** with amines followed by deprotection of the diethyl phosphonate with TMSBr gave amides **2e–i**.

We next turned our attention to the naphthalene analogs since these compounds generally had more favorable clogD values. For instance, using the commercial software from ACD, the clog*D*s for **2b** and **3b** were calculated to be −2.28 and −0.8, respectively. Phosphonate **3a**, which was reported previously to have a K_i of 179 μM against PTP1B,[13] was the starting point for further SAR investigation. Incorporation of the *ortho*-Br moiety and addition of a methyl group at the 7-position resulted in a dramatic improvement in potency, giving **3b** with an IC_{50} of 330 nM against PTP1B. The compound was also significantly more potent than the corresponding quinoline analog **2b**. Attempts to improve intrinsic activity by extending to the secondary binding site, as was described above for the quinoline analogs, also resulted in compounds with substantial improvement in enzymatic potency but negligible oral absorption (results not shown). As a result, we focused on small groups in hoping to improve potency while maintaining oral bioavailability. Compounds **3b–f** were synthesized according to Scheme 2.

Reaction of $LiP(OEt)_2$ with aldehyde **10** gave alcohol **11**, which was oxidized under the Swern conditions to the corresponding ketone. The ketone was treated with an excess of DAST to give the phosphonate **12**, which was processed to **3b** as mentioned above. Compound

Table 1. Potency and selectivity of naphthalene- and quinoline-difluoromethylphosphonate PTP1B inhibitors

Compound	X	R	IC_{50}[a] (μM) (FDP assay)	
			PTP1B	CD45
1a	H		0.016	50
1b	H		2.0	>50
1c	Br		0.12	>50
2a	H	Me	>50	>50
2b	Br	Me	14.0	>50
2c	Br		0.16	>50
2d	Br		0.007	37.8
2e	Br		0.060	>50
2f	Br		0.027	>50
2g	Br		0.045	>50
2h	Br	C(O)NHMe	1.26	>50
2i	Br	$C(O)NH_2$	0.25	>50
3a	H	H	K_i = 179[b]	na
3b	Br	Me	0.33	>50
3c	Br	$CH_2NHC(O)Me$	1.5	>50
3d	Br	$CH_2S(O)_2Me$	1.30	>50
3e	Br	CH_2OMe	0.51	>50
3f	Br	CH_2CN	0.09	26.8
3g	Br	CN	0.12	>50
4	—	—	0.31	>50

[a] Values are averages from 2 to 8 titrations, all compounds show similar activities against T-cell phosphatase and the values are not included here.
[b] Literature value; na, not available.

12 was treated with *N*-bromosuccinamide (NBS) in the presence of a catalytic amount of benzoyl peroxide to give bromide **13**, which was reacted with a variety of nucleophiles and then processed to **3c–f** as shown.

As shown in Table 1, polar groups such as acetamide (**3c**) and methylsulfone (**3d**) were detrimental to intrinsic potency. Less polar groups such as CH_2OMe (**3e**) and CH_2CN (**3f**) were tolerated. Compound **3f** was potent against PTP1B with an IC_{50} of 90 nM. It also showed reasonable plasma exposure in mice after oral dosing (C_{max} = 6 μM at 2 h with 20 mg/kg dosing). The nitrile analog **3g**, however, had the best overall profile in terms of potency and oral bioavailability. It had an IC_{50} of

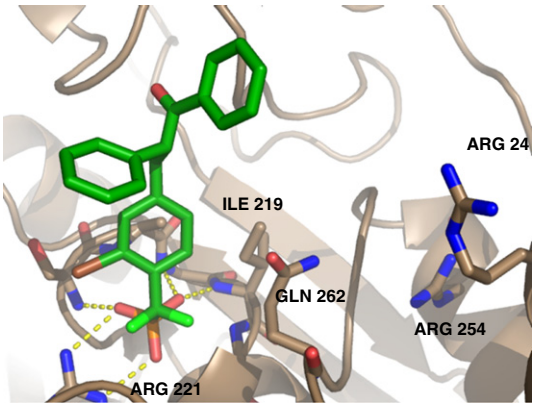


Figure 2. High resolution (1.6 Å) X-ray structure of the PTP1B-**1c** complex. Active site residues important for binding are labeled in black and the structure of compound **1c** is in green.

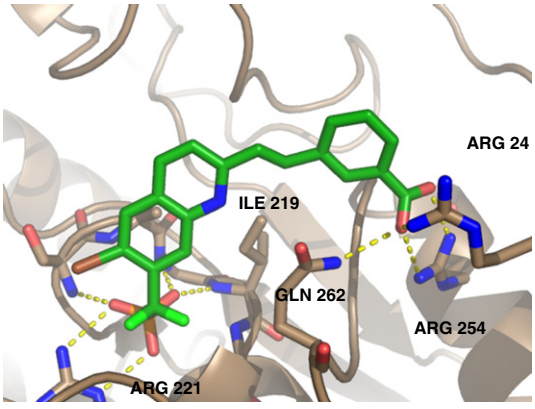


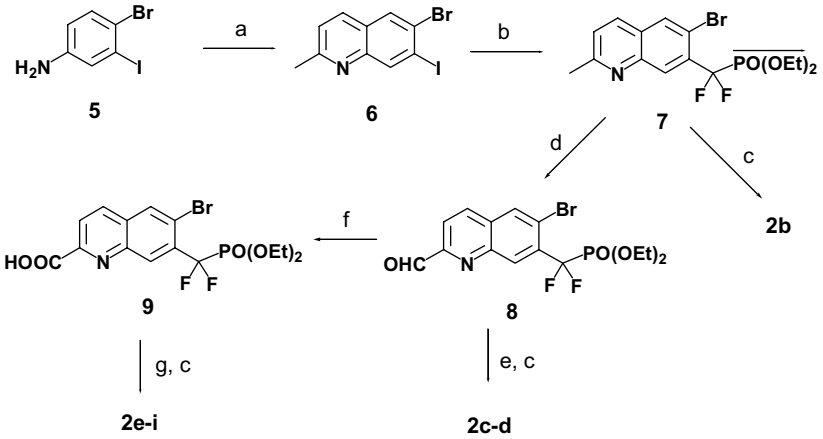
Figure 3. Compound **2d** modeled into the active site of PTP1B. The ligand scaffold is in green. The difluoromethylphosphonate in both **1c** and **2d** binds to the primary binding site similarly.

120 nM in the enzymatic assay and was the most active compound in inhibiting yeast growth among a panel of potent phosphonate inhibitors with comparable inhibitory potencies against the PTP1B enzyme.[14] It also exhibited good pharmacokinetic profiles in several species as shown in Table 2.

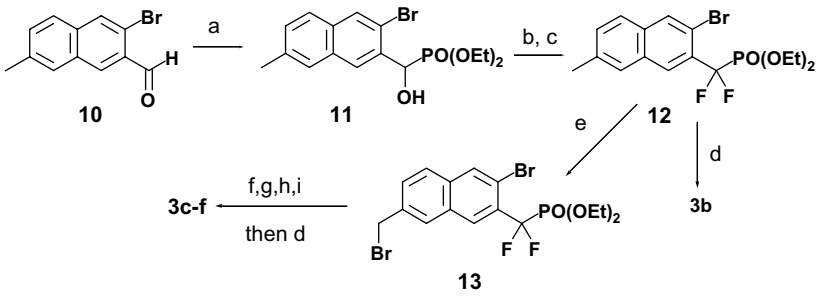
In diet-induced obese (DIO) mice, **3g** exhibited good oral bioavailability ($F = 24\%$), slow clearance (CL = 0.71 mL/kg/min), and good elimination half live ($t_{1/2} = 6$ h). The oral bioavailability in higher species such as rats ($F = 4\%$) and squirrel monkeys ($F = 2\%$) were substantially lower but excellent exposures were achieved with oral dosing as shown in Table 2. In addition, **3g** had very low clearance rate in rats and squirrel monkeys, resulting in very long elimination $t_{1/2}$s (>17 and >30 h, respectively). The corresponding *regio* isomer **4** also exhibited good pharmacokinetic profiles in mice but was 3-fold less potent.

Compound **3g** and **4** were synthesized according to Scheme 3. 6,7-Dibromo-2-naphthonitrile (**14**) was prepared from commercially available 4,5-dibromo-*o*-xylene in two steps according to literature procedures.[15] Monolithiation with *n*-BuLi in the presence of TMSCl followed by treating the resultant TMS derivatives with iodine monochloride (ICl) gave a mixture of 6-bromo-7-iodo- and 7-bromo-6-iodo-2-naphthonitrile (**15** and **16**). The mixture was reacted with ((diethoxyphosphinyl)-difluoromethyl)zinc bromide using CuBr as the catalyst to yield a mixture of diethyl difluoromethyl-phosphonates **17** and **18** which was separated by normal phase HPLC using a Zorbax silica column. Deprotection of **17** and **18** with TMSBr followed by treating the TMS-esters with water gave the corresponding phosphonic acid **3g** and **4**. The free acids were easily converted to the diammonium salts by treating with an excess of aqueous ammonia in MeOH. The experimental procedures for making **3g** and **4** are described in note.[16]

Compound **3g** was studied in animal models of diabetes and cancer. For example, in oral glucose tolerance tests (oGTT) in DIO mice,[17] **3g** exhibited dose dependent inhibition (60%, 80% and 100% inhibition at 1, 3 and 10 mg/kg, respectively) of glucose excursion when given orally 2 h before oral glucose challenge with an esti-



Scheme 1. Reagents and conditions: (a) chloranil, concd HCl in *i*-PrOH at reflux, then crotonaldehyde in *i*-PrOH via syringe pump; (b) $(EtO)_2P(O)CF_2ZnBr$, CuBr, DMF, 60 °C; (c) TMSBr, DCM, rt and then co-evaporate with water/ethanol (3×); (d) SeO_2, ethanol, reflux; (e) $ArCH_2P^+Ph_3Br^-$, LiHMDS, THF, −78 °C—rt; (f) AgO, MeOH, rt; (g) EDCI, RNH_2, TEA, trace DMAP, DCM, rt.

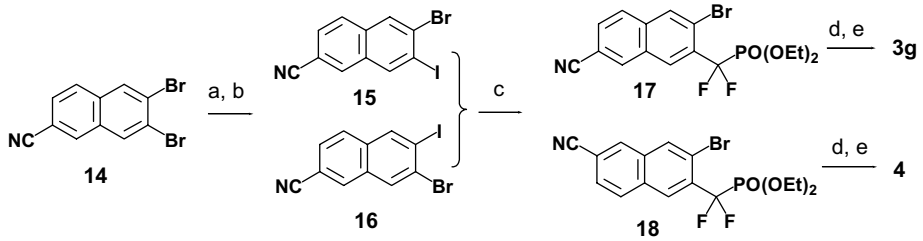


Scheme 2. Reagents and conditions: (a) $(EtO)_2PH$, LiHMDS, THF, −78 °C; (b) $(COCl)_2$, DMSO TEA, DCM, −78 °C—rt; (c) DAST, chloroform, rt; (d) TMSBr, DCM, rt and then co-evaporate with water/ethanol; (e) NBS, cat. Benzoyl peroxide, CCl_4, reflux; (f) **3c**: aq NH_3, then EDCI, AcOH; (g) **3d**: MeSNa in MeOH, then oxone®, acetone; (h) **3e**: NaOMe, MeOH; (i) **3f**: NaCN, MeOH.

Table 2. Pharmacokinetic profiles of **3g** in mice, rats and monkeys

Species	Dose[a] (mg/kg) iv/po	F (%)	$t_{1/2}$ (h)	CL (mL/min/kg)	$C_{max}/C_{24\ h}$ (μM)
DIO mice	5/20[b]	24	6	0.71	17.4/9.0
Rat	5/20[c]	4	>17	0.03	75.6/45.3
Squirrel monkey	2/10[c]	2	>30	0.02	24.0/19.0

[a] The diammonium salt was used for PK studies.
[b] Dosed as a solution in 0.9% NaCl for iv and as a 25% PEG200 solution for po.
[c] Dosed as a solution in 0.9% NaCl for iv and as a 0.5% methylcellulose solution for po.



Scheme 3. Reagents and conditions: (a) *n*-BuLi, TMSCl, THF, −78 °C; (b) ICl, DCM, rt; (c) $(EtO)_2P(O)CF_2ZnBr$, CuBr, DMF, 45 °C; (d) TMSBr, DCM, rt and then co-evaporate with water/ethanol (3×); (e) NH_4OH, MeOH, rt.

mated ED_{50} of 0.8 mg/kg. The anticancer effect of **3g** was studied in the NDL2 *Ptpn1* transgenic mice.[3] When dosed orally at 30 mg/kg for 21 days, **3g** caused a significant delay in the onset of tumor development in NDL2 $Ptpn1^{+/+}$ mice when compared to vehicle treatment, extending the median tumor free days (T50) from 28 days to 75 days. Interestingly, the blood glucose levels of the drug treated animals in this study were lowered to comparable levels seen in the NDL2 $Ptpn1^{-/-}$ animals, further demonstrating the antidiabetic potential of this PTP1B inhibitor.

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16. Experimental procedures for making compound **3g**. (a) *7-Bromo-6-iodo-2-naphthonitrile (**15**) and 6-bromo-7-iodo-2-naphthonitrile (**16**):* To a solution of 6,7-dibromo-2-naphthonitrile (15 g) and TMSCl (6.73 mL) in THF (250 mL) at −78 °C was added *n*-BuLi (53 mL, 1.6 M in hexanes, precooled to −20 °C) rapidly with vigorous stirring and the mixture was stirred for an additional 5 min and quenched with saturated aq NH_4Cl. The mixture was then extracted with ethyl acetate and the organic layer was washed with water and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated and the crude was purified by flash column chromatography to give the desired product as a mixture of two regioisomers. ^{1}H NMR (400 MHz, acetone-d_6) (a mixture of two regioisomers): δ 8.53 (s, 1H), 8.42 (s, 1H), 8.33 (s, 1H), 8.30 (s, 1H), 8.23 (s, 1H), 8.20 (s, 1H), 8.18 (d, 1H), 8.07 (d, 1H), 7.82–7.77 (m, 2H), 0.50 (s, 18H). To a solution of the above product in dichloromethane (250 mL) was added excess ICl and the mixture was stirred at rt for 1 h. The solution was washed with 10% $Na_2S_2O_3$ until all ICl was consumed. The solution was then washed with water, brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated and the residue was recrystallized from ether/hexanes to give the desired product. ^{1}H NMR (400 MHz, acetone-d_6) (a mixture of two regioisomers): δ 8.74 (s, 1H), 8.73 (s, 1H), 8.46–8.44 (m, 4H), 8.10–8.07 (m, 2H), 7.84–7.81 (m, 2H); (b) *Compound **17** and **18**:* A flame dried round-bottomed flask was charged with CuBr (99.999%) and THF (10 mL), followed by ((diethoxyphosphinyl)difluoromethyl)zinc bromide (29 mL, 1.72 M in THF) following the procedure of Shibuya et al.[10] The suspension was stirred under N_2 for 15 min. A mixture of **15** and **16** (7.1 g) was then added as a solid and the mixture was heated to 45 °C overnight and cooled to rt. The suspension was then quenched with half saturated NH_4Cl and extracted with 1:1 ether/ethyl acetate (3×). The extracts were processed as usual to give the crude product which was first purified by flash chromatograph (40% ethyl acetate in hexanes). The two regioisomers were then separated by normal phase HPLC using a Zorbax silica column. Eluting with 50% ethyl acetate/hexanes first gave the less polar isomer **17**. ^{1}H NMR (400 MHz, acetone-d_6): δ8.72 (s, 1H), 8.54 (s, 1H), 8.46 (s, 1H), 8.19 (d, 1H), 7.95 (d, 1H), 4.26 (m, 4H), 1.33 (t, 6H). Continued elution gave the more polar isomer **20**. ^{1}H NMR (400 MHz, acetone-d_6): δ 8.56 (s, 2H), 8.43 (s, 1H), 8.35 (d, 1H), 7.93 (d, 1H), 4.26 (m, 4H), 1.33 (t, 6H). The regiochemistry of the two isomers was vigorously established by COSY, NOSEY, HSQC, and HMBC experiments; (c) *Compound **3g**:* A solution of diethyl [(3-bromo-7-cyano-2-naphthyl)(difluoro)methyl]phosphonate (2.2 g) in dichloromethane (5 mL) and TMSBr (7 mL) was stirred at rt overnight and concentrated. The residue was co-evaporated with dichloromethane (2×), ethanol/water (2×) and then dissolved in 20 mL of methanol. Ammonia (30%) was then added with vigorous stirring and the mixture was concentrated and co-evaporated with methanol (3×). The solid residue was washed with ether to give the desired product as a white powder. ^{1}H NMR (400 MHz, acetone-d_6): δ 8.65 (s, 1H), 8.58 (s, 1H), 8.54, (s, 1H), 8.16 (d, J = 5 Hz, 1H), 7.90 (d, J = 5 Hz, 1H). MS (−ESI): m/z 360.1 and 362.0 $(M-1)^-$.
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