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# Synthesis and Biological Activity of 3,3-Diamino-sulfonylacrylonitriles as Novel Inhibitors of Glucose Induced Insulin Secretion from Beta Cells

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**Abstract**—Pinacidil analogues, for example, *N*-cyano-*N*′-(3,5-dichlorophenyl)-*N*″-(3-methylbutyl)guanidine, **1**, have previously been described as potassium channel openers on beta cells and smooth muscle cells. In the present study 3,3-diamino-sulfonylacrylonitrile, a new bioisostere of the cyanoguanidine group, was investigated. 3,3-Diamino-sulfonylacrylonitriles were prepared in a two step synthesis from the corresponding isothiocyanates and sulfonylacetonitriles. Single crystal X-ray crystallography and NMR spectroscopy were used to establish the structure of 2-(4-chlorophenylsulfonyl)-3-cyclobutylamino-3-(3,5-dichlorophenylamino)-acrylonitrile **3i**. The analysis confirmed that **3i** assumes a staggered conformation considered as the energetically most favourable. The compounds synthesised have been identified as potent inhibitors of glucose stimulated insulin secretion from beta cell lines and rat pancreatic islets with minimal effects on vascular smooth muscle.


## Introduction

Insulin is of essential importance in controlling glucose and lipid homeostasis. Abnormal insulin secretion can lead to metabolic diseases such as obesity and diabetes. In type 2 diabetes insulin resistance associated with hypersecretion of insulin leads to beta cell failure, and will ultimately necessitate insulin treatment. Even before onset of type 2 diabetes, beta cell function is impaired, characterized by lack of first phase insulin secretion and insulin pulsatility. This impairment could contribute to the degeneration of beta cells, which finally will lead to overt type 2 diabetes.[1,2] Reduction of insulin secretion to induce beta cell rest has been shown to prevent beta cell failure[3] and to prevent the development of type 2 diabetes in animal models.[4,5] It has furthermore been suggested that beta cell rest can be applicable in treatment of type 1 diabetes[6] and certain forms of obesity.[7,8]

Inhibition of insulin release can be achieved by activation of ATP sensitive potassium ($K_{ATP}$) channels of pancreatic beta cells. $K_{ATP}$ channels are present not only in beta cells but also in other cell types, such as smooth muscle, in neurons of the central nervous system, in the heart and in skeletal muscle.[9,10] The potassium channel opener diazoxide, which activates the $K_{ATP}$ channels of beta cells and smooth muscles, has been used to inhibit insulin release in vitro and in vivo. Diazoxide is used to treat insulin hypersecretion from insulin producing tumours,[11] and persistent hyperinsulinaemic hypoglycemia of infancy (PHHI)[12] and also experimentally, to treat obesity[13] and type 1 diabetes.[6] The use of diazoxide is however associated with severe side effects such as oedema and hypertrichosis, which largely can be related to the vasodilating properties of the compound.[14] By combining structural elements from diazoxide and pinacidil, a cyanoguanidine derivate which activates $K_{ATP}$ channels of smooth muscle, it has been possible to generate new compounds, which potently inhibit insulin release without significant effects on smooth muscle.[15–18]

In analogy, pinacidil-like cyanoguanidine derivatives, for example, *N*-cyano-*N*′-(3,5-dichlorophenyl)-*N*″-(3-methylbutyl)guanidine (**1**, Fig. 1), have recently been characterized as selective and potent inhibitors of insulin

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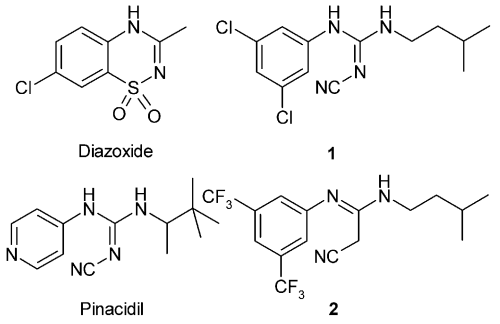



**Figure 1.** Activators of ATP sensitive potassium channels.

release from beta cell lines and rat pancreatic islets.[19] To further explore structural analogues of pinacidil as inhibitors of insulin release, we developed a solid-phase synthesis of cyanoacetamidines to prepare compound libraries by parallel solid phase synthesis.[20]

The screening of these libraries showed that certain *N*-[3,5-bis(trifluoromethyl)phenyl]-*N'*-alkylcyanoacetamidines (e.g., **2**) were weak activators of $^{86}Rb^{+}$ efflux from beta cells (data not shown) suggesting that these compounds could be optimized to generate potent activators of pancreatic $K_{ATP}$ and thereby inhibit insulin release. While investigating the solution phase synthesis of these cyanoacetamidines, we found that sulfonylacrylonitriles **3** (Fig. 2, Scheme 1) were readily accessible. The structure activity relationship of this class of compounds was examined to identify that certain 3,3-diamino-sulfonylacrylonitriles are potent inhibitors of glucose stimulated insulin release with minimal dilatation of vascular smooth muscles.

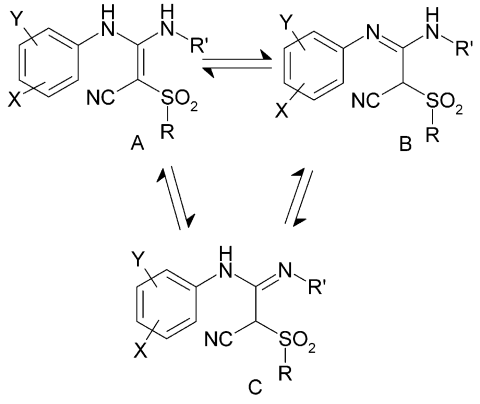



**Figure 2.** Tautomeres of **3**.

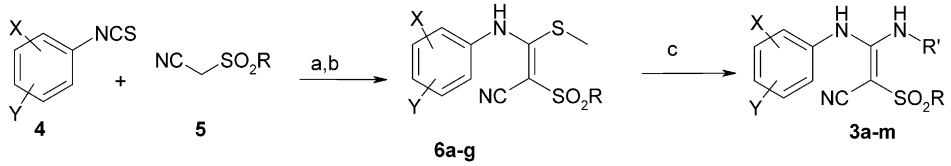
The 3,3-diamino-sulfonylacrylonitriles can exist in different tautomeric forms, which furthermore can adopt several conformational isomers. NMR spectroscopy and single crystal X-ray crystallography have been used to show that the present sulfonylacrylonitriles exemplified by compound **3i** predominantly exist as tautomer A (Fig. 2) and in a staggered conformation.

## Chemistry

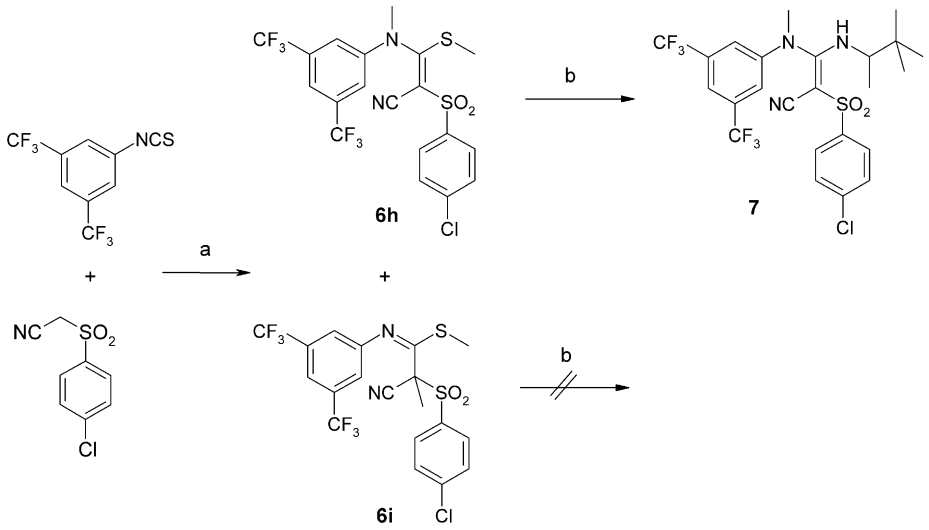
The sulfonylacrylonitriles **3** were synthesized according to Scheme 1. Aromatic isothiocyanates (**4**) were added to the sulfonylacetonitriles (**5**) in the presence of potassium carbonate. Upon removal of excess potassium carbonate the resulting salts were S-alkylated with 3 equivalents of methyl iodide, to give the methylsulfanylsulfonylacrylonitriles (**6**) in moderate to high yields. The methylsulfide group was substituted with excess alkylamines to produce the desired sulfonylacrylonitriles **3** in variable yields. Generally, more bulky amines resulted in lower yields. For the preparation of **6**, the filtration to remove the excess potassium carbonate was critical. When methyl iodide was added without filtration in an attempt to prepare 3-[3,5-bis(trifluoromethyl)phenyly;amino]-2-(4-chlorophenylsulfonyl)-3-methylsulfanylacrylonitrile (**6b**) two products **6h** and **6i** were isolated and separated by crystallization (Scheme 2). Treatment of the *N*-methylated compound **6h** with 1,2,2-trimethylpropylamine gave 3-[*N*-(3,5-bis(trifluoromethyl)phenyl)-*N*-methylamino]-2-(4-chlorophenylsulfonyl)-3-(1,2,2-trimethylpropyl-amino)acrylonitrile (**7**). In contrast, **6i**, which was a result of methylation of the carbon between the electron withdrawing cyano- and sulfonyl group, proved resistant to the 1,2,2-trimethylpropylamine treatment.

## Structure Analysis

Three tautomers, A, B, and C, can be considered for the sulfonylacrylonitriles **3a**–**m** (Fig. 2). The $^{1}H$ NMR spectrum of **3i** determined in DMSO showed a broad proton signal at 9.42 (NH) and a broad doublet at 8.27 (NH) suggesting that the sulfonylacrylonitrile (**3i**) exists as the tautomer A. The geometry of the double bond could however not be determined by $^{1}H$ NMR or NOE experiments but had to be solved by single crystal structure determination as shown in Figure 3. The configuration of the two phenyl groups with respect to the C(2)–C(3) double bond is (*E*). Relevant geometrical information is provided in Table 1. All interatomic bond lengths conform to values commonly observed for




**Scheme 1.** Reagents and conditions: (a) $K_2CO_3$, acetone, filtration; (b) MeI; (c) $R'NH_2$, 80–100 °C, 17–40 h.




**Scheme 2.** Reagents and conditions: (a) $K_2CO_3$, acetone, 2 h then MeI; (b) 1,2,2-trimethylpropylamine, 80 °C, 68 h.

chemically similar structures.[21] The angles have nearly ideal values with a few exceptions. The bond angles at the $sp^2$ hybridised carbon atoms C(2) and C(3) are distorted by up to ±3° from 120° for both atoms, see Table 1. The smallest angles are the branching angles of the olefinic bond, S(1)–C(2)–C(1) and N(2)–C(3)–N(3). This probably occurs to diminish the steric hindrance between neighbouring 3,5-dichlorophenyl and cyclobutyl groups, and between 4-chlorophenyl and 3,5-dichlorophenyl groups. As a consequence the olefin is not planar but has opening angles S(1)–C(2)–C(3)–N(2) of 30.81(12)° and C(1)–C(2)–C(3)–N(3) of 19.4(13).

Probably as a result of lone-pair repulsion the O(1)–S(1)–O(2) angle is large, 119.31(5)°, whereas in contrast, the C(2)–S(1)–C(11) angle is as small as 103.66(5)° A moderate *intra*-molecular hydrogen bond exists between N(2)–H(N2) and O(1) with the N(2).....O(1) = 2.8247(12) Å, H(N2)....O(1) = 2.16(3) Å, and the angle N(2)-H(N2).....O(1) = 135(2)°.

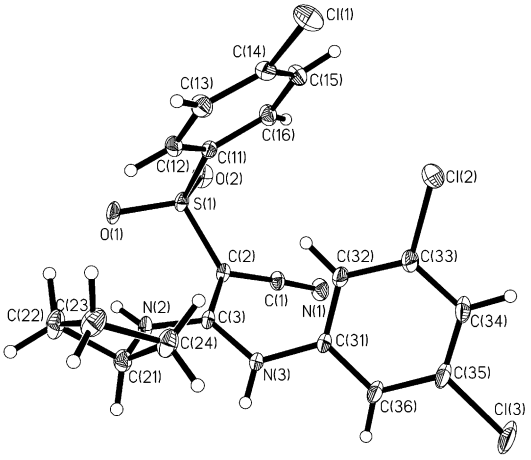



**Figure 3.** XP[22] drawing with 50% probability thermal ellipsoids for all non-hydrogen atoms of compound (**3i**). The hydrogen atoms are shown as open circles for clarity.

Two weak *intra*-molecular hydrogen bonds existing between atoms C(12)–H(12).....O(1) and C(16)–H(16).....O(2) may assist in supporting the large O(1)–S(1)–O(2) angle. In the crystal structure only one moderate *inter*-molecular hydrogen bond exists to ensure the crystalline cohesion, namely the bond between N(3)–H(N3) of molecule I at (x, y, z) and N(1) of molecule II at (3/2−x, 1/2+y, 1/2−z) with N(3)....N(1) = 2.8967(14)Å, H(N3)....N(1) = 2.06(3) Å, and N(3)–H(N3)....N(1) = 171(2)°. Two weak *inter*-molecular hydrogen bonds which also may assist in supporting the three-dimensional framework, are found between C(15)–H(15) of molecule I and N(1) of molecule III at (1−x, −y, −z) and between C(34)–H(34) of molecule I and O(1) of molecule IV at (1/2+x, 1/2−y, −1/2+z).

## Results and Discussion

The synthesized compounds were evaluated for their ability to inhibit glucose stimulated insulin release from the βTC6 rat beta cell line. The smooth muscle activity of the compounds was meassured by the relaxation of

**Table 1.** Relevant geometrical data of **3i**

| Bond | Length in Å | Bond angle | Angle in° |
|---|---|---|---|
| N(1)–C(1) | 1.1616 (12) | N(1)–C(1)–C(2) | 177.26 (11) |
| C(1)–C(2) | 1.3987 (12) | O(2)–S(1)–O(1) | 119.31 (5) |
| C(2)–C(3) | 1.4159 (13) | O(2)–S(1)–C(2) | 108.17 (5) |
| C(2)–S(1) | 1.7304 (9) | O(1)–S(1)–C(2) | 109.11 (5) |
| S(1)–O(2) | 1.4364 (9) | O(2)–S(1)–C(11) | 108.38 (5) |
| S(1)–O(1) | 1.4461 (8) | O(1)–S(1)–C(11) | 107.10 (5) |
| S(1)–C(11) | 1.7654 (10) | C(2)–S(1)–C(11) | 103.66 (5) |
| C(3)–N(2) | 1.3273 (11) | C(1)–C(2)–C(3) | 119.55 (8) |
| C(3)–N(3) | 1.3579 (12) | C(1)–C(2)–S(1) | 117.49 (7) |
| N(2)–C(21) | 1.4585( 13) | C(3)–C(2)–S(1) | 122.35 (6) |
| N(3)–C(31) | 1.4120 (12) | C(2)–C(3)–N(2) | 121.51 (8) |
| | | C(2)–C(3)–N(3) | 120.78 (8) |
| | | N(2)–C(3)–N(3) | 117.67 (8) |
| | | C(3)–N(2)–C(21) | 125.50 (8) |
| | | C(3)–N(3)–C(31) | 125.24 (8) |

**Table 2.** Structure and biological data for sulfonylacrylonitriles **3a**–**3m, 7,** diazoxide and pinacidil

| Compds | X,Y | R′ | R | Inhibition of insulin release βTC6 cells[a] | | Relaxation of rat aorta rings[b] $IC_{50}$, μM |
|---|---|---|---|---|---|---|
| | | | | $IC_{50}$, μM | Efficacy (%) | |
| **3a** | 5-pyridyl | $CH(CH_3)C(CH_3)_3$ | 4-Cl-Ph | NA | | 3.4±3.3 |
| **3b** | 3-$CF_3$,5-$CF_3$ | (*S*)-$CH(CH_3)C(CH_3)_3$ | 4-Cl-Ph | 0.83±0.6 | 58±3.36 | >300 |
| **3c** | 3-$CF_3$,,5-$CF_3$ | (*R*)-$CH(CH_3)C(CH_3)_3$ | 4-Cl-Ph | 0.13±0.1 | 80±5.5 | >300 |
| **3d** | 3-$CF_3$,5-$CF_3$ | $CH_2CH_2CH_3$ | 4-Cl-Ph | 0.19±0.04 | 69±4,18 | 108±5.8 |
| **3e** | 3-$CF_3$,5-$CF_3$ | $CH_2CH_2CH(CH_3)_2$ | 4-Cl-Ph | 1.06±0.4 | 75±5.1 | 3.6±2.3 |
| **3f** | 3-$CF_3$,5-$CF_3$ | (*R*/*S*)-$CH(CH_3)C(CH_3)_3$ | $CH_3$ | 1.06±0.3 | 54±4.7 | 47±11.0 |
| **3g** | 3-Cl,5-Cl | (*R*/*S*)-$CH(CH_3)C(CH_3)_3$ | 4-Cl-Ph | 0.82±0.1 | 44±8.1 | 102±70.4 |
| **3h** | 3-Cl,5-Cl | $C(CH_3)_2CH_2CH_3$ | 4-Cl-Ph | 0.18±0.1 | 73±5.7 | 195±70.9 |
| **3i** | 3-Cl,5-Cl | Cyclobutyl | 4-Cl-Ph | 1.43±0.6 | 70±6.0 | 7±6.9 |
| **3j** | 3-$OCH_3$,5-$OCH_3$ | $C(CH_3)_2CH_2CH_3$ | 4-Cl-Ph | 0.59±0.4 | 65±7.5 | 17±12.9 |
| **3k** | 3-$OCH_3$,5-$OCH_3$ | Cyclobutyl | 4-Cl-Ph | 1.73±0.8 | 71±4.9 | 237±124 |
| **3l** | 3-$OCH_3$,5-$OCH_3$ | (*R*/*S*)-$CH(CH_3)C(CH_3)_3$ | $CH_3$ | NA | | 11.1±3.4 |
| **3m** | 3,4-$OCH_2O$ | $C(CH_3)_2CH_2CH_3$ | 4-Cl-Ph | 0.84±0.2 | 56±4.8 | 130±55.6 |
| **7** | 3-$CF_3$,5-$CF_3$- | (*R*/*S*)-$CH(CH_3)C(CH_3)_3$ | 4-Cl-Ph | NA | | >300 |
| Diazoxide | | | | 22.98±4.1 | 25±3.8 | 12.8±2.5 |
| Pinacidil | | | | >100 | | 0.8±0.2 |

NA: not active. Values are means±SEM calculated from at least 3 measurements.
[a]Inhibition of glucose stimulated insulin release from βTC6 cells.
[b]Relaxation of phenylephrin induced contraction of rat aorta rings.

phenylephrin contracted rat aorta rings (Table 2). Initially the effect of changing the *N*-cyanoguanidine group of pinacidil to a 3,3-diamino-sulfonylacrylonitrile was investigated. The pinacidil analogue **3a** relaxed phenylephrin contracted aorta rings ($EC_{50}$ = 3.4±3.3 μM) only about 5 times less potent than pinacidil ($EC_{50}$ = 0.8±0.2 μM) but was without effects on insulin secretion. Changing the pyridine ring to disubstituted phenyl rings gave rise to compounds, which were able to suppress insulin secretion in analogy with what was previously described for phenylcyanoguanidine derivatives.[19] Both the (*R*) and (*S*) enantiomers of 3,5-bis(trifluoromethyl)phenyl sulfonylacrylonitriles (**3b** and **3c**) carrying the pinacidil side chain inhibited insulin secretion from βTC6-cells with similarly high potency and with no activity on aorta rings. The propyl as well as the 3-methylbutyl derivatives **3d** and **3e** both potently inhibited insulin release. Whereas **3d** had only minimal activity on smooth muscle **3e** potently relaxed phenylephrin contracted aorta rings ($EC_{50}$ = 3.6±2.3 μM). In this series, the size and the stereo chemistry of the alkylamino side-chain therefore seems to be of little importance for beta cells activity. When the 4-chlorophenylsulfonyl group was changed to methylsulfonyl (**3f**), the activity remained unchanged (Table 2).

The *N*-methylated compound **7** did not show any activity on either beta cells or smooth muscle, emphasizing the importance of an acidic hydrogen atom in this position for biological activity. Changing the trifluoromethyl groups to chlorine atoms and keeping the 4-chlorophenylsulfonyl group resulted in compounds, which were active on the beta cells. The effect of the alkyl group modifications on smooth muscle activity was pronounced in this series. The pinacidil side chain and the *tert*-amyl group (**3g** and **3h**) afforded potent inhibitors of insulin release with little or no smooth muscle activity. In case of the cyclobutyl derivative (**3i**) beta cell selectivity was reduced due to an increase in smooth muscle activity. Changing the aromatic substituents from 3,5-bis(trifluoromethyl)- or 3,5-dichloro- to 3,5-dimethoxy- slightly reduced the beta cell activity of compound **3j**–**3k** whereas the methanesulfonyl derivative of the dimethoxy series (**3l**) was inactive on beta cells but active on smooth muscle. Similarly the 3,4-methylenedioxy derivative **3m** was only slightly less potent with respect to inhibition of insulin release from beta cells as the corresponding 3,5-dichloro derivative **3h**. To further characterize the effects of the sulphonylacrylonitriles, selected compounds were evaluated on freshly isolated rat pancreatic islets.[19] Both compounds **3h** ($IC_{50}$ = 0.32±0.2 μM) and **3m** ($IC_{50}$ = 0.95±0.2 μM) inhibited glucose stimulated insulin release considerably more than diazoxide ($IC_{50}$ = 20.3±8.8 μM).

The staggered conformation for **3i** found in the crystal form (Fig. 3), which is stabilized by intra molecular hydrogen bonding between alkylamino N–H and an oxygen of the sulfonyl group, corresponds to one of the two energetically most favourable conformations found for cyanoguanidines.[23] The hydrochloride of the vaso-relaxant potassium channel opener AL0670 (Fig. 4) has

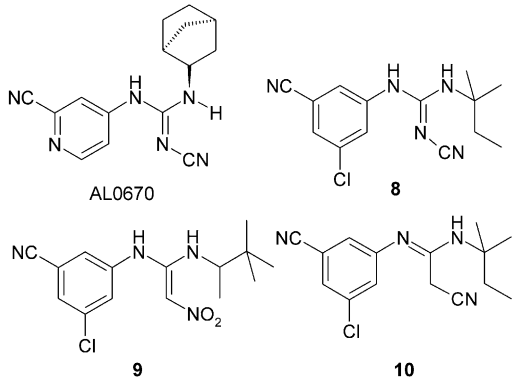



**Figure 4.** Structures of reference compounds.

been shown by X-ray crystallography to adopt one of the favorable conformations analogous to the one adopted by **3i**.[24] In contrast, pinacidil has been shown to exist in the conformation shown in Figure 1 in the crystal form.[25] Compound **2,** which has been found to be in the cyanoacetamidine form[20] (Fig. 1) and not in the acrylonitril form, was inactive with respect to inhibition of insulin release from βTC6 cells and relaxation of phenylephrin contracted aorta rings (data not shown) The inactivity of this compound confirms the findings of Yoshiizumi et al.[26] who found that, by using NMR spectroscopy, the predominant tautomers of the corresponding diaminocyanoguanidine (**8**) and nitroethendiamine (**9**), were as shown in Figure 4. Compounds **8** and **9** were able to relax smooth muscles. The hybrid of these two compounds, **10**, in contrast were found to exist as a cyanoacetamidine, which was inactive on smooth muscle cells.[26] This further supports the importance of an acidic NH-group for the activity of these compounds.

## Conclusion

*N*-Alkyl, *N*-aryl-3,3-diamino-sulfonylacrylonitriles have been synthesized. When the *N*-cyano group of pinacidil was changed to the bioisostere sulfonylacetonitril, the activity on smooth muscle was retained. Further modification of the structure to yield *N*-alkyl, *N*-3,5-disubstituted phenyl-3,3-diaminosulfonylacrylonitriles resulted in new potent and efficacious inhibitors of glucose induced insulin secretion from βTC6 cells. With respect to smooth muscle relaxation the structure activity relation is less clear. In the 3,5-bis(trifluoromethyl)-phenylamino sulfonylacrylonitrile series substantial variation of the alkylamino group was tolerated giving potent and beta cell selective compounds.

## General procedures

### Chemistry

Reagents, starting materials and solvents were purchased from common commercial suppliers and were used as received. Acetone was dried over night over molecular sieves (0.3 nm). Evaporation was carried out on a rotary evaporator at bath temperatures $<40\,^{\circ}C$ and under appropriate vacuum. Flash chromatography was carried out on a Biotage flash 40 using Biotage flash columns (KP SIL, 60Å particle size 32–63 μM). Melting points were determined with a Büchi B545 apparatus and are uncorrected. Proton NMR spectra were recorded at ambient temperature using a Bruker Avance DPX 200 (200 MHz) and Bruker Avance DPX 300 (300 MHz) with tetramethylsilane as internal standard. Chemical shifts are given in ppm (δ) and splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; quint, quintet; sext, sextet; sept, septet; m, multiplet, and br = broad. The 70 eV E.I. solid mass spectra were recorded on a Finnigan MAT-TSQ 70 mass spectrometer. Reactions were followed by thin layer chromatography performed on silica gel 60 F254 (Merck) or ALUGRAM®SIL G/$UV_{254}$ (MACHEREY-NAGEL) TLC aluminium sheets. Elemental analyses (C, H, N) were performed by Novo Nordisk, Microanalytical Laboratory, Denmark.

**2-(4-Chlorophenylsulfonyl)-3-methylsulfanyl-3-(pyridin-3-ylamino)acrylonitrile (6a).** To a solution of 4-chlorophenylsulfonylacetonitrile (1.00 g, 4.6 mmol) in dry acetone (10 mL) first dry potassium carbonate (1.28 g, 9.3 mmol) and then pyridin-3-yl isothiocyanate (0.663 g, 4.9 mmol) were added. The resulting mixture was stirred at room temperature under nitrogen for 4 h, and then filtered. To the filtrate, methyl iodide (0.315 mL, 5.1 mmol) was added. The mixture was stirred at room temperature for 16 h. The reaction mixture was concentrated and the residue was taken up into ethyl acetate and water. The organic layer was washed twice with 1 N aqueous HCl. The organic phase was dried ($Na_2SO_4$) and concentrated. The residue was purified by flash chromatography using EtOAc/heptane 2:1 as eluent and recrystallization in EtOAc to give 294 mg (16%) of the title compound. Mp 181–182 °C. $^1$H NMR (300 MHz, $CDCl_3$): δ = 2.20 (s, 3H), 7.36 (dd, 1H), 7.54 (dm, 2H), 7.6 (m, 1H), 7.88 (dm, 2H), 8.55 (m, 2H), 9.85 (br s, 1H); EI SP/MS: 365 (M+).

**2-(4-Chlorophenylsulfonyl)-3-(pyridin-3-ylamino)-3-(1,2,2-trimethylpropylamino)acrylonitrile (3a). 6a** (0.186 g, 0.5 mmol) was stirred in 1,2,2-trimethyl propylamine (1 mL) for 22 h at 100 °C under nitrogen. The reaction mixture was concentrated. The residue was dissolved in DCM, washed with water, dried ($Na_2SO_4$) and concentrated. The crude product was purified by flash chromatography using EtOAc as eluent to give 124 mg (58%) of the title compound as syrup, which could be crystallized from EtOAc/heptane 2:1 to give 65 mg (30%). Mp 172–174 °C. $^1$H NMR (300 MHz, $CDCl_3$): δ = 0.85 (s, 9H), 0.9 (d, 3H), 3.03 (m, 1H), 7.35 (m, 2H), 7.48 (d, 2H), 7.80 (d, 2H), 8.33 (br s, 1H), 8.5 (br s, 1H). Anal. calcd for $C_{20}H_{23}ClN_4O_2S$; C 57.34%, H 5.53%, N 13.37%; found C 57.28%, H 5.62%, N 13.21%.

**3-[3,5-Bis(trifluoromethyl)phenylamino]-2-(4-chlorophenylsulfonyl)-3-methylsulfanylacrylonitrile (6b).** To a solution of 4-chlorophenylsulfonylacetonitrile (2.00 g, 9.3 mmol) in dry acetone (20 mL) first dry potassium carbonate (2.56 g, 18.5 mmol) and then 3,5-bis(trifluoromethyl)phenyl isothiocyanate 2.64 g, 9.7 mmol) were added. The resulting mixture was stirred at room temperature under nitrogen for 4.5 h, and then filtered. To the filtrate, methyl iodide (1.72 mL, 27.8 mmol) was added. The mixture was stirred at room temperature for 2 h. The pH was adjusted to ~4 with 1 N HCl and water was added. The precipitated syrup was isolated by decantation and was crystallized in EtOAc/heptane 1:2 to give the title compound as white crystals in 82% (3.81 g) yield. Mp 150.5–152.5 °C $^1$H NMR (300 MHz, $CDCl_3$): δ = 2.25 (s, 3H), 7.57 (d, 2H), 7.79 (s, 2H), 7.83 (s, 1H), 7.88 (d, 2H), 10.0 (br s, 1H). Anal. calcd for $C_{18}H_{11}ClF_6N_2O_2S_2$: C 43.17%, H 2.21%, N 5.59%. Found: C 43.25%, H 2.16%, N 5.59%.

**(*S*)-3-[3,5-Bis(trifluoromethyl)phenylamino]-2-(4-chlorophenylsulfonyl)-3-(1,2,2-trimethylpropylamino)acrylonitrile (3b). 6b** (0.200 g, 0.4 mmol) was stirred in (*S*)-

1,2,2-trimethylpropylamine (0.2 mL) at 80 °C in a sealed flask for 20 h. The reaction mixture was concentrated and the residue dissolved in DCM, washed twice with 1 N aqueous HCl and once with water. The organic phase was dried (sodium sulfate) and concentrated. The residue was crystallized from EtOAc/heptane 1:2 to give 25 mg (11%) of the title compound as colourless crystals. Mp 159–161.5 °C. $^1$H NMR (300 MHz, $CDCl_3$): δ = 0.95 (s, 9H), 1.15 (d, 3H), 3.00 (m, 1H), 7.40 (s, 2H), 7.50 (d, 2H), 7.70 (s, 1H), 7.82 (d, 2H). Anal. calcd for $C_{23}H_{22}ClF_6N_3O_2S$: C 49.87%, H 4.00%, N 7.59%. Found: C 49.59%, H 4.09%, N 7.36%.

**(*R*)-3-[3,5-Bis(trifluoromethyl)phenylamino]-2-(4-chlorophenylsulfonyl) - 3 - (1,2,2 - trimethylpropylamino)acrylonitrile (3c). 6b** (0.300 g, 0.6 mmol) was stirred in 1,2,2-trimethylpropylamine (1 mL) for 17 h at 75 °C under nitrogen. Work-up as described for **3b** gave 0.161 g (48%) of the title compound. Mp 164–165 °C. $^1$H NMR (200 MHz, $CDCl_3$): δ = 0.90 (s, 9H), 1.00 (d, 3H), 3.04 (m, 1H), 7.36 (s, 2H), 7.48 (d, 2H), 7.67 (s, 1H), 7.80 (d, 2H). Anal. calcd for $C_{23}H_{22}ClF_6N_3O_2S$: C 49.87%, H 4.00%, N 7.59%. Found: C 49.95%, H 4.15%, N 7.48%.

**3-[3,5-Bis(trifluoromethyl)phenylamino]-2-(4-chlorophenylsulfonyl)-3-propylaminoacrylonitrile (3d). 6b** (0.40 g, 0.80 mmol) was stirred in *n*-propylamine (1.0 mL) at 75 °C in a sealed flask for 19 h. Work-up as described for **3b** gave 266 mg (65%) of the title compound as white crystals. Mp 196.5–198.5 °C. $^1$H NMR (300 MHz, $CDCl_3$): δ = 0.90 (t, 3H), 1.55 (p, 2H), 2.88 (q, 2H), 7.40 (s, 2H), 7.48 (d, 2H), 7.68 (s, 1H), 7.81 (d, 2H). Anal. calcd for $C_{20}H_{16}ClF_6N_3O_2S$: C 46.93%, H 3.15%, N 8.21%. Found: C 46.82%, H 3.19%, N 8.10%.

**3-[3,5-Bis(trifluoromethyl)phenylamino]-2-(4-chlorophenylsulfonyl)-3-(3-methylbutylamino) acrylonitrile (3e).** 6b (0.40 g, 0.80 mmol) was stirred in 3-methylbutylamine (2 mL) at 80 °C for 18 h under nitrogen. Work-up as described for **3b** except that the crude product was purified by flash chromatography using EtOAc/heptane 1:3 before crystallization to give 235 mg (55%) of the title compound as colorless crystals. Mp 159.5–161 °C. $^1$H NMR (300 MHz, $CDCl_3$): δ = 0.80 (d, 6H), 1.38 (q, 2H), 1.52 (m, 1H), 2.90 (q, 2H), 7.40 (s, 2H), 7.48 (d, 2H), 7.69 (s, 1H), 7.80 (d, 2H). Anal. calcd for $C_{22}H_{20}ClF_6N_3O_2S$: C 48.94%, H 3.73%, N 7.78%. Found: C 49.15%, H 3.73%, N 7.67%.

**3-[3,5-Bis(trifluoromethyl)phenylamino]-2-methanesulfonyl-3-methylsulfanylacrylonitrile (6c).** To a solution of methanesulfonylacetonitrile (0.55 g, 4.6 mmol) in dry acetone (10 mL) first dry potassium carbonate (1.28 g, 9.3 mmol) and then 3,5-bis(trifluoromethyl)phenyl isothiocyanate (1,32 g, 4.8 mmol) were added. The resulting mixture was stirred at room temperature under nitrogen for 135 min. before filtration. To the filtrate, methyl iodide (0.86 mL, 13.9 mmol) was added. The mixture was stirred at room temperature for 2 h. Then pH was adjusted to ~4 with 1 N aqueous HCl. The liquid was decanted off to leave the precipitated syrup. The syrup was crystallized from EtOAc/heptane 1:2 to give 413 mg (22%) of the title compound. Mp 129–131 °C. $^1$H NMR (200 MHz, $CDCl_3$): δ = 2.33 (s, 3H), 3.24 (s, 3H), 7.72 (s, 2H), 7.80 (s, 1H), 9.95 (br s, 1H); EI SP/MS: 404 ($M^+$).

**3-[3,5-Bis(trifluoromethyl)phenylamino]-2-methanesulfonyl-3-(1,2,2-trimethyl-propylamino)acrylonitrile (3f). 6c** (0.225 g, 0.56 mmol) was stirred in 1,2,2-trimethylpropylamine (1 mL) for 22 h at 100 °C under nitrogen in a sealed flask. Work-up as described for **3b**. The residue was crystallized from EtOAc/heptane 1:4 to give 146 mg (57%) of the title compound. Mp 184.5–188.5 °C. $^1$H NMR (200 MHz, $CDCl_3$): δ = 0.9 (s, 9H), 1.03 (d, 3H), 3.0 (m, 1H), 7.52 (s, 2H), 7.68 (s, 1H); EI SP/MS: 457 ($M^+$). Anal. calcd for: $C_{18}H_{21}F_6N_3O_2S$: C 47.26%, H 4.63%, N 9.19%. Found : C 47.50%, H 4.66%, C 9.14%.

**2-(4-Chlorophenylsulfonyl)-3-(3,5-dichlorophenylamino)-3-methylsulfanylacrylonitrile (6d).** To a solution of 4-chlorophenylsulfonylacetonitrile (2.00 g, 9.3 mmol) in dry acetone (20 mL) first dry potassium carbonate (2.56 g, 18.6 mmol) and then 3,5-dichlorophenyl isothiocyanate (1.98 g, 9.8 mmol) were added. The resulting mixture was stirred at room temperature under nitrogen for 17 h, and then filtered. To the filtrate, methyl iodide (1.72 mL, 27.8 mmol) was added. The mixture was stirred at room temperature for 45 min. Then the pH was adjusted to 2 with 1 N aqueous HCl. The precipitate was filtered off and washed with water to give 3.75 g (93%) of the crude product. Recrystallization from EtOAc/heptane 1:1 gave 3.46 g (86%) of the title compound. Mp 128–131 °C $^1$H NMR (200 MHz, $CDCl_3$): δ = 2.25 (s, 3H), 7.20 (d, 2H), 7.30 (t, 1H), 7.55 (d, 2H), 7.85 (d, 2H), 9.80 (br s, 1H); EI SP/MS: 434 ($M^+$), 436 ($M^{+2}$).

**2-(4-Chlorophenylsulfonyl)-3-(3,5-dichlorophenylamino)-3-(1,2,2-trimethylpropylamino)acrylonitrile (3g). 6d** (0.347g, 0.8 mmol) was stirred in 1,2,2-trimethyl propylamine (1 mL) for 22 h at 100 °C under nitrogen in a sealed flask. Work-up as described for **3b** gave 235 mg (60%) of the title compound. Mp 163–169 °C. $^1$H NMR (200 MHz, $CDCl_3$): δ = 0.9 (s, 9H), 1.0 (d, 3H), 3.05 (m, 1H), 6.85 (br s, 2H), 7.2 (br s, 1H), 7.50 (d, 2H), 7.78 (d, 2H); EI SP/MS: 485 ($M^+$), 487 ($M^{+2}$), 489 ($M^{+4}$), 491 ($M^{+6}$). Anal. calcd for: $C_{21}H_{22}Cl_3N_3O_2S$: C 51.81%, H 4.55%, N 8.63%. Found: C 51.93%, H 4.75%, N 8.32%.

**2-(4-Chlorophenylsulfonyl)-3-(3,5-dichlorophenylamino)-3-(1,1-dimethylpropylamino)acrylonitrile (3h). 6d** (0.359 g, 0.8 mmol) was stirred in 1,2,2-trimethylpropylamine (0.125 mL) for 22 h at 50 °C and for 18 h at 80 °C under nitrogen in a sealed flask. Work-up as described for **3b** except that the crude product was purified by flash chromatography using EtOAc/heptane 1:2 before crystallization to give 64 mg (17%) of the title compound. Mp 189–191.5 °C (EtOAc). $^1$H NMR (200 MHz, $CDCl_3$): δ = 0.99 (t, 3H), 1.38 (s, 6H), 1.68 (q, 2H), 6.26 (br s, 1H), 6.50 (d, 2H), 7.10 (t, 1H), 7.55 (dt, 2H), 7.81 (dt, 2H); EI SP/MS: 475 ($M^{+4}$), 473 ($M^{+2}$), 471 ($M^+$). Anal. calcd for $C_{20}H_{20}Cl_3N_3O_2S$: C 50.81%, H 4.26%, N 8.89%. Found: C 50.46%, H 4.21%, N 8.64%.

**2-(4-Chlorophenylsulfonyl)-3-cyclobutylamino-3-(3,5-dichlorophenylamino)acrylonitrile (3i). 6d** (0.90 g, 2.1 mmol), cyclobutylamine (0.50 mL, 6.3 mmol) and acetonitrile (2 mL) were stirred for 40 h at 100 °C under nitrogen in a sealed flask. Work-up as described for **3i** gave 0.47 g (49%) of the title compound. 179.5–181.5 °C (EtOAc). $^1$H NMR (200 MHz, $CDCl_3$): $\delta = 1.65$ (m, 2H), 1.95 (m, 2H), 2.1 (m, 2H), 3.65 (sext, 1H), 6.88 (d, 2H), 7.18 (t, 1H), 7.47 (d, 2H), 7.78 (d, 2H). $^1$H NMR (200 MHz, DMSO-$d_6$): $\delta = 1.55$ (m, 2H), 2.1 (m, 4H), 3.98 (sextet, 1H), 6.65 (d, 2H), 7.24 (t, 1H), 7.72 (s, 4H), 8.27 (br d, NH), 9.42 (br s, NH); EI SP/MS: 459 ($M^+4$), 357 ($M^+2$) 455 ($M^+$). Anal. calcd for $C_{19}H_{16}Cl_3N_3O_2S$: C 49.96%, H 3.53%, N 9.20%; found: C 50.10%, H 3.57%, N 9.13%.

**2-(4-Chlorophenylsulfonyl)-3-(3,5-dimethoxyphenylamino)-3-methylsulfanylacrylonitrile (6e).** To a solution of 4-chlorophenylsulfonylacetonitrile (1.00 g, 4.6 mmol) in dry acetone (10 mL) first dry potassium carbonate (1.28 g, 9.3 mmol) and then 3,5-dimethoxyphenyl isothiocyanate (0.96 g, 4.9 mmol) were added. The resulting mixture was stirred at room temperature under nitrogen for 135 min, and then filtered. To the filtrate, methyl iodide (0.86 mL, 13.9 mmol) was added. The mixture was stirred at room temperature for 4 h. The reaction mixture was concentrated. The residue was dissolved in DCM, washed with water, dried ($Na_2SO_4$) and concentrated. The residue was crystallized from EtOAc/heptane 1:2 to give 1.27 g (65%) of the title compound. Mp 119.5–123.0 °C. $^1$H NMR (200 MHz, $CDCl_3$): $\delta = 2.29$ (s, 3H), 3.80 (s, 6H), 3,36 (m, 3H), 7.53 (d, 2H), 7.87 (d, 2H), 9.82 (br s, 1H); EI SP/MS: 424 ($M^+$).

**2-(4-Chlorophenylsulfonyl)-3-(3,5-dimethoxyphenylamino)-3-(1,1-dimethylpropylamino) acrylonitrile (3j). 6e** (1.05 g, 2.5 mmol) was stirred in 1,1-dimethylpropylamine (0.87 mL, 7.5 mmol) and acetonitrile (4 mL) for 17 h at 100 °C in a sealed flask under nitrogen. Work-up as described for **3b** gave 0.42 g (36%) of the title compound. Mp 189–190 °C. $^1$H NMR (200 MHz, $CDCl_3$): $\delta = 0.95$ (t, 3H), 1.35 (s, 6H), 1.65 (q, 2H), 3.62 (s, 6H), 5.88 (d, 2H), 6.20 (t, 1H) 6.45 (br s, 1H), 7.0 (br s, 1H), 7.50 (dt, 2H), 7.83 (dt, 2H). Anal. calcd for $C_{22}H_{26}ClN_3O_4S$: C 59.96%, H 5.65%, N 9.06%. Found: C 56.82%, H 5.64%, N 8.90%.

**2-(4-Chlorophenylsulfonyl)-3-(3,5-dimethoxyphenylamino)-3-cyclobutylaminoacrylonitrile (3k). 6e** (0.99 g, 2.4 mmol), cyclobutylamin (0.60 mL, 7.2 mmol) and acetonitrile (4 mL) was stirred for 17 h at 100 °C in a sealed flask under nitrogen. Work-up as described for **3b** gave 0.90 g (83%) of the title compound. Mp 163.5–164.5 °C. $^1$H NMR (200 MHz, $CDCl_3$): $\delta = 1.6$ (m, 2H), 1.85 (m, 2H), 2.1 (m, 2H), 3.77 (s, 6H), 3.8 (m, 1H), 6.12 (d, 2H), 6.32 (t, 1H), 7.47 (dt, 2H), 7.81 (dt, 2H); MA calcd for $C_{21}H_{22}ClN_3O_4S$: C 56.31%, H 4.95%, N 9.38%. Found: C 56.40%, H 4.99%, N 9.30%.

**3-(3,5 - Dimethoxyphenylamino) - 2 - methanesulfonyl - 3-methylsulfanylacrylonitrile (6f).** To a solution of methanesulfonylacetonitrile (0.55 g, 4.6 mmol) in dry acetone (10 mL) first dry potassium carbonate (1.28 g, 9.3 mmol) and then 3,5-dimethoxyphenyl isothiocyanate (0.96 g, 4.9 mmol) were added. The resulting mixture was stirred at room temperature under nitrogen for 23 h, filtered and washed with ethanol. To the filtrate, methyl iodide (0.86 mL, 13.9 mmol) was added. The mixture was stirred at room temperature for 4 h. The reaction mixture was filtered and the filtrate was concentrated. The residue was dissolved in dichloromethane and washed with water. The organic layer was dried ($Na_2SO_4$) and concentrated. The residue was recrystallized from ethyl acetate to give the title compound (0.728 g, 48%). Mp 158–159.5 °C; $^1$H NMR (200 MHz, $CDCl_3$): $\delta = 2.27$ (s, 3H), 3.19 (s, 3H), 3.80 (s, 6H), 6.48 (t, 1H), 6.44 (d, 2H) EI SP/MS: 328 ($M^+$).

**3-(3,5 - Dimethoxyphenylamino) - 2 - methanesulfonyl - 3-(1,2,2-trimethylpropylamino)acrylonitrile (3l). 6f** (0.263 g, 0.8 mmol) was stirred in 1,2,2-trimethylpropylamine (1 mL) for 17 h at 75 °C under nitrogen. Work-up as described for **3i** gave the title compound as syrup, 262 mg (86%), which crystallizes after standing at room temperature. Mp 151.5–152.5 °C. $^1$H NMR (200 MHz, $CDCl_3$): $\delta = 0.85$ (s, 9H), 1.01 (d, 3H), 3.12 (s, 3H), 3.26 (m, 1H), 3.78 (s, 6H), 6.25 (d, 2H), 6.35 (t, 1H); EI SP/MS: 381 (M+). Anal. calcd for $C_{18}H_{27}N_3O_4S$: C 56.67%, H 7.13%, N 11.01%. Found: C 56.84%, H 7.26%, N 10.82%.

**3-(Benzo[1,3]dioxol-5-ylamino)-2-(4-chlorophenylsulfonyl)-3-methylsulfanylacrylonitrile (6g).** To a solution of 4-chlorophenylsulfonylacetonitrile (5.15 g, 23.9 mmol) in dry acetone (50 mL) first dry potassium carbonate (1.28 g, 9.3 mmol) and then 3,4-methylenedioxyphenyl isothiocyanate (6.61 g, 47.8 mmol) were added. The resulting mixture was stirred at room temperature under nitrogen for 60 h, and then filtered. To the filtrate, methyl iodide (4.43 mL, 71.8 mmol) was added. The mixture was stirred at room temperature for 1.5 h. Then the pH was adjusted to 1 with 1 N aqueous HCl. The precipitate was filtered off and washed with water to give 9.18 g (94%) of the title compound. Mp 196–200 °C $^1$H NMR (200 MHz, $CDCl_3$): $\delta = 2.22$ (s, 3H), 6.05 (s, 2H), 6.68 (m, 2H), 6.80 (d, 1H), 7.53 (d, 2H), 7.87 (d, 2H), 9.80 (br s, 1H); EI SP/MS: 408 ($M^+$).

**3 - (Benzo[1,3]dioxol - 5 - ylamino) - 3 - (1,1 - dimethylpropyl amino)-2-(4-chloro-phenylsulfonyl)acrylonitrile (3m). 6g** (0.327g, 0.8 mmol) was stirred in 1,2,2-trimethylpropyl amine (1 mL) for 17 h at 100 °C under nitrogen in a sealed flask. Work-up as described for **3b** gave 81 mg (26%) of the title compound. Mp 169–170 °C. $^1$H NMR (200 MHz, $CDCl_3$): $\delta = 0.95$ (t, 3H), 1.37 (s, 6H), 1.65 (q, 2H), 5.98 (s, 1H), 6.2 (m, 2H), 6.45 (br s, 1H), 6.66 (d, 1H), 6.98 (br s, 1H), 7.50 (d, 2H), 7.80 (d, 2H). Anal. calcd for $C_{21}H_{22}ClN_3O_4S$ ($\times 0.5H_2O$): C 55.20%, H 5.07%, N 9.20%; Found: C 55.18%, H 4.90%, N 8.90%.

**3-[*N*-[3,5-Bis(trifluoromethyl)phenyl]-*N*-methylamino]-2-(4-chlorophenylsulfonyl)-3-methylsulfanyl acrylonitrile (6 h) and *N*-[3,5-Bis(trifluoromethyl) phenyl]-2-(4-chlorobenzenesulfonyl)-2-cyano-2-methylthioacetimidic acid**

**methyl ester (6i).** To a solution of 4-chlorophenylsulfonylacetonitrile (1.10 g, 5.1 mmol) in dry acetone (12 mL) first dry potassium carbonate (1.41 g, 10.2 mmol) and then 3,5-bis(trifluoromethyl)phenyl isothiocyanate (1.44 g, 5.3 mmol) were added. The resulting mixture was stirred at room temperature under nitrogen. After 2 h methyl iodide (3.80 mL, 61.2 mmol) was added. The mixture was stirred at room temperature for 18 h, followed by filtration and concentration. Crystallization from EtOAc/heptane 1:3 gave 0.52 g (20%) of compound **6h**. $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 2.40$ (s, 3H), 3.65 (s, 3H), 7.36 (s, 2H), 7.42 (d, 2H), 7.58 (s, 1H), 7.65 (d, 2H); ); EI SP/MS: 514 (M+). From the mother liquor another crop of crystals was obtained, to give **6i**. 1.60 g (60%); $^1$H NMR (300 MHz, CDCl3): $\delta = 1.94$ (s, 3H), 2.16 (s, 3H), 7.18 (s, 2H), 7.63 (d + s, 3H), 8.02 (d, 2H). ); EI SP/MS: 514 (M+).

**3-[*N*-[3,5-Bis(trifluoromethyl)phenyl)-*N*-methylamino]-2-(4-chlorophenylsulfonyl)-3-(1,2,2-trimethylpropylamino)-acrylonitrile (7).** To a solution of **6h** (0.300 g, 0.58 mmol) in dry acetonitrile (2 mL) was added 1,2,2-trimethylpropylamine (0.12 g) and dry triethylamine (89 μL). The mixture was stirred for 68 h at 80 °C under nitrogen. The reaction mixture was concentrated. Purification by flash chromatography using EtOAc/heptane 1:4 and 1:3 as eluent gave a syrup (142 mg) which was crystallized from ethanol/water to give 110 mg (33%) of the title compound. Mp 151.5–154 °C. $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 0.90$ (s, 9H), 1.10 (d, 3H), 3.10 (m, 1H), 3.30 (s, 3H), 7.10 (s, 2H), 7.46 (s, 1H), 7.55 (d, 2H), 7.85 (d, 2H), 8.10 (br d, 1H). Anal. calcd for $C_{24}H_{24}ClF_6N_3O_2S$: C 50.75%, H 4.26%, N 7.40%, Cl 6.24%. Found: C 50.79%, H 4.28%, N 7.24%, Cl 6.19%.

## X-ray crystallography

2-(4-Chlorophenylsulfonyl)-3-cyclobutylamine-3-(3,5-dichlorophenylamino)-acrylonitrile (**3i**), was dissolved in ethanol and the solution was left to evaporate slowly at room temperature. Large single crystals appeared. One specimen was selected and subjected to X-ray diffraction experiments on a Nonius kappa CCD diffractometer while cooled with a nitrogen cryo-stream. X-ray diffraction data were collected using Collect software[27] in ω-scan mode with thin slicing, allowing a three dimensional integration of the collected reflection intensities. Details of the data collection and crystal data are given in Table 3. The unit cell obtained from 225 reflections in the angular range in θ from 4.0° to 23.3° conformed to a monoclinic crystal system.[28] The data were reduced using the Maxus software.[29] From the intensity distribution the space group was determined to be $P2_1/n$ with one molecule in the asymmetric unit using XPREP.[22] A numerical absorption correction method was used.[30] The structure was solved and refined with SHELXTL.[22,31] The direct methods located the 4-chlorophenylsulfonyl group and the remaining chlorine atoms in the first cycle. Subsequent difference Fourier syntheses revealed remaining atoms. All hydrogen atoms were clearly visible in the difference electron density map calculated after introduction of anisotropic thermal displacement parameters for all non-hydrogen atoms. The hydrogen atomic positions were allowed to refine independently, but their thermal displacement parameters were fixed at 0.05 Å$^2$. The final agreement factors are $R(F) = 5.22\%$ for 301 parameters refined against 11734 observed reflections ($F_o^2 > 2\sigma(F_o^2)$). The two largest electron density difference peaks of 1.8 and 1.4 e/Å$^3$ are close to the chlorine atoms Cl(2) and Cl(3). Remaining electron density difference peaks are lower

**Table 3.** Crystal data and structure refinement for **3i**

| | | |
|---|---|---|
| Empirical formula | $C_{19}H_{16}Cl_3N_3O_2S$ | |
| Formula weight | 456.76 | |
| Temperature | 122(1) K | |
| Crystal system | Monoclinic | |
| Space group | $P2_1/n$ | |
| Unit cell dimensions | $a = 12.118(2)$ Å | $\alpha = 90°$. |
| | $b = 10.294(2)$ Å | $\beta = 93.73(3)°$. |
| | $c = 16.302(3)$ Å | $\gamma = 90°$. |
| Volume | 2029.3(7) Å$^3$ | |
| $Z$ | 4 | |
| Density (calculated) | 1.495 Mg/m$^3$ | |
| F(000) | 936 | |
| Wavelength (Mo $K_\alpha$) | 0.71073 Å | |
| Absorption coefficient μ (Mo $K_\alpha$) | 0.575 mm$^{-1}$ | |
| Crystal size | 0.06×0.14×0.23 mm$^3$ | |
| $\theta$ Range for data collection | 2.50 to 42.01°. | |
| Index ranges | $-22 \le h \le 22$, $-19 \le k \le 19$, $-30 \le 1 \le 30$ | |
| Reflections collected | 162287 | |
| Independent reflections | 14161 [R(int) = 0.0434] | |
| Completeness to $\theta = 42.01°$ | 99.8% | |
| Absorption correction | Numerical | |
| Max. and min. transmission | 0.930 and 0.828 | |
| Refinement method | Full-matrix least-squares on $F^2$ | |
| Data/restraints/parameters | 14161 / 0 / 301 | |
| Goodness-of-fit on $F^2$ | 1.148 | |
| Final R indices [I > 2σ(I)] | $R(F) = 0.0522$, $wR(F_o^2) = 0.1161$ | |
| R indices (all data) | $R(F) = 0.0678$, $wR(F_o^2) = 0.1247$ | |
| Largest diff. peak and hole | 1.810 and −1.709 e.Å$^{-3}$ | |

than 0.7 e/Å$^3$. The largest negative electron density peaks are also close to chlorine. This may be indicative of anharmonic vibration of chlorine. Molecular geometry: a plane was approximated through all six atoms of the olefin, and the angles of the substituents with this plane were calculated as follows: 4-chlorophenyl group 86.27(4)°, 3,5-dichlorophenyl 63.68(4)°, cyclobutyl 67.85(6)°. The 3,5-dichlorophenyl moiety makes an angle of 70.30(5)° with the 4-chlorophenyl moiety plane. Torsion angles of special interest:

**Torsion angles at the double bond.** S1–C2–C3–N2 30.81°(0.12), S1–C2–C3–N3 −151.38° (0.07), C1–C2–C3–N2 −158.40°(0.09), C1–C2–C3–N3 19.41°(0.13). **Torsion angles at the phenyl rings**: C2–S1–C11–C12 −90.87° (0.08), C2–S1–C11–C16 84.04° (0.08), C3–N3–C31–C32 31.39°(0.14), C3–N3–C31–C36 −153.23° (0.10). **Weak hydrogen bonds**. *Intra*-molecular distance C(12)-H(12)....O(1) 2.9384(14) Å, angle C(12)–H(12)....O(1) 104(2)°, distance C(16)–H(16).....O(2) 2.9717(15) Å, angle C(16)–H(16).....O(2) 105(2)°. *Inter*-molecular: distance C(15)–H(15).... N(1) of molecule III at (1–*x*, –*y*, –*z*) 3.3802(15) Å, angle C(15)–H(15).... N(1) 170(2)°, distance C(34)–H(34)....O(1) of molecule IV at (1/2 + x, 1/2−y, −1/2 + z) 3.2681(14) Å, angle C(34)–H(34).....O(1) 150(2). Full crystallographic data for the structure in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary material.[32]

### Inhibition of glucose stimulated insulin release from βTC6 cells

βTC6 cells[33] were cultured at $5\times10^4$ cells/microtiter well in DMEM + 10% FCS, 11 mM glucose, 1% Glutamax and 20 mM Hepes for three days (95% humidity, 37 °C, 5% $CO_2$). Cells were washed twice with NN buffer (all in mM: NaCl 114; KCl 47; $KH_2PO_4$, 1.21; $MgSO_4$, 1.16; $NaHCO_3$, 25.5; CaCl, $2H_2O$, 2.5; HEPES, 10) supplemented with 0.1% BSA and incubated 60 min in this buffer. All wells were aspirated and the cells incubated for 3 h with NN buffer, 22 mM glucose, 0.1 mM IBMX and serial dilutions of the compounds. A reference compound, NNC 55-2006,[16] served as positive control. A test for responsiveness towards a series of glucose concentrations was included in every assay to ensure functionality. The supernatant from each well was harvested and insulin content was measured by an in-house ELISA using guinea pig anti-insulin antibodies and a rat insulin as standard. The results were analysed in Prism (Graphpad Software, San Diego, CA, USA) and expressed as $IC_{50}$ and $E_{max}$ (the maximum inhibition obtained at 10 μM of the compound). SEM was calculated for all compounds.

### Inhibition of glucose induced insulin release in Wistar rat islets

Islets were isolated by Collagenase and gradient centrifugation in Ficoll gradient (40–13%). Isolated islets were incubated in bulk overnight in RPMI, 10% FCS, 11 mM glucose. The islets were handpicked and placed at 10 islets / microtiter well and cultured overnight in DMEM, 10% FCS, 3 mM glucose. Essentially the islets were tested as described for the βTC6 but with no addition of IBMX. The insulin content was measured in the same ELISA as used for the βTC6 cells.

### Relaxation of rat aorta rings

Female Wistar rats weighing approximately 150–200 g were killed by cervical dislocation, and the thoracic aorta was removed. The aorta was cut into rings of approximately 5 mm wide. Ring preparations were mounted in 5 mL (Danish Myo Technology, Aarhus, Denmark) or 10 mL organ baths (Schuler Organ Bath 809, Hugo Sachs Elektronik, Germany) with a resting tension of 2 g, and bathed in Krebs Ringer solution with the following composition (mM): NaCl 118.5; $NaHCO_3$ 25.0; KCl 4.7; $CaCl_2$ 6.8; $MgCl_2$ 2.4; and glucose 11.1 in double distilled water. The ringer solution was continuously aerated with 95% $O_2$/5% $CO_2$ at 37 °C. Cumulative concentration response curves (0.1–300 μM) were constructed for all test compounds on top of a pre-contraction induced by 0.3 μM phenylephrine. All test compounds were freshly dissolved in dimethylsulfoxide. Potency of a compound ($EC_{50}$), with regard to producing smooth muscle relaxation, was defined as the concentration required obtaining a half maximal dilating effect. $EC_{50}$ was estimated for individual concentration response curves by four parameter non-linear, logistic regression using Myodata software (Danish Myo Technology, Aarhus, Denmark).

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