Journal Pre-proof

Design, synthesis, and biological evaluation of aryl piperazines with potential as antidiabetic agents via the stimulation of glucose uptake and inhibition of NADH:ubiquinone oxidoreductase

R. Devine, M. Kelada, S. Leonard, D.S.D. Martin, J.M.D. Walsh, C.J. Breen, R.B. Driver, G.K. Kinsella, J.B.C. Findlay, J.C. Stephens

PII: S0223-5234(20)30387-1

DOI: https://doi.org/10.1016/j.ejmech.2020.112416

Reference: EJMECH 112416

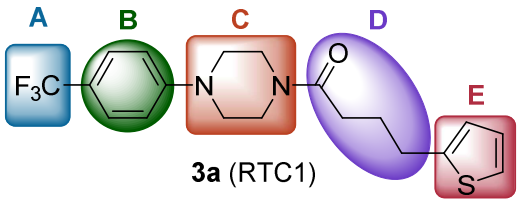
To appear in: *European Journal of Medicinal Chemistry*

Received Date: 23 August 2019

Revised Date: 29 April 2020

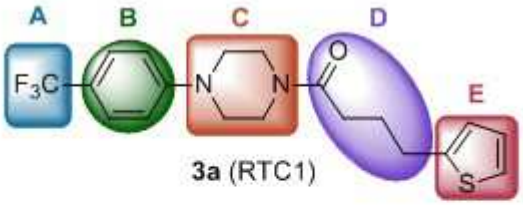
Accepted Date: 29 April 2020

Please cite this article as: R. Devine, M. Kelada, S. Leonard, D.S.D. Martin, J.M.D. Walsh, C.J. Breen, R.B. Driver, G.K. Kinsella, J.B.C. Findlay, J.C. Stephens, Design, synthesis, and biological evaluation of aryl piperazines with potential as antidiabetic agents via the stimulation of glucose uptake and inhibition of NADH:ubiquinone oxidoreductase, *European Journal of Medicinal Chemistry* (2020), doi: https://doi.org/10.1016/j.ejmech.2020.112416.



3a (RTC1)

- 2.6 fold increase in glucose uptake
- NADH:ubiquinone oxidoreductase inhibition(IC_{50} = 27 μM)
- Positive *in vitro* DMPK and *in vivo* PK results
- 41 derivatives of **3a** (RTC1) evaluated with modification at sites A-E



3a (RTC1)

- 2.6 fold increase in glucose uptake
- NADH:ubiquinone oxidoreductase inhibition(IC_{50} = 27 μM)
- Positive *in vitro* DMPK and *in vivo* PK results
- 41 derivatives of **3a** (RTC1) evaluated with modification at sites A-E

Title

Design, synthesis, and biological evaluation of aryl piperazines with potential as antidiabetic agents via the stimulation of glucose uptake and inhibition of NADH:ubiquinone oxidoreductase.

Authors

R. Devine,[a, †] M. Kelada,[a,†] S. Leonard,[a,b,†] D. S. D. Martin,[b] J. M. D. Walsh,[a] C. J. Breen,[b,c] R. B. Driver,[a] G. K. Kinsella,[d] J. B. C. Findlay,[b,e] J. C. Stephens.[a,c,*]

a Department of Chemistry, Maynooth University, Maynooth, Co. Kildare, Ireland
b Department of Biology, Maynooth University, Maynooth, Co. Kildare, Ireland
c Maynooth University Human Health Research Institute, Maynooth University, Maynooth, Co. Kildare, Ireland
d School of Food Science and Environmental Health, College of Sciences and Health, Technological University Dublin, Dublin D07 ADY7, Ireland
e School of Biochemistry and Molecular Biology, University of Leeds, Leeds, UK
† Joint first author, alphabetical order
* Corresponding author

Key words
Diabetes, glucose uptake, complex 1, piperazine, pharmacokinetics, structure activity relationship

Highlights

- Forty one aryl piperazines derivatives have been generated and tested *in vitro* for their ability to stimulate glucose uptake.
- Six of the compounds were reported to effectively stimulate glucose uptake *in vitro* and inhibit NADH:ubiquinone oxidoreductase.
- The hit compound **3a** (RTC1) remained the most efficacious with a 2.57 fold increase in glucose uptake compared to vehicle control and micromolar inhibition of NADH:ubiquinone oxidoreductase (IC_{50} = 27 µM).
- Preliminary i*n vitro* DMPK and *in vivo* PK studies suggested that **3a** (RTC1) would not provoke adverse drug-drug interactions, yet be readily metabolised, avoid rapid excretion, with a short half-life, and have good tissue distribution.

Abstract

The management of blood glucose levels and the avoidance of diabetic hyperglycemia are common objectives of many therapies in the treatment of diabetes. An aryl piperazine compound **3a** (RTC1) has been described as a promoter of glucose uptake, in part through a cellular mechanism that involves inhibition of NADH:ubiquinone oxidoreductase. We report herein the synthesis of 41 derivatives of **3a** (RTC1) and a systematic structure-activity-relationship study where a number of compounds were shown to effectively stimulate glucose uptake *in vitro* and inhibit NADH:ubiquinone oxidoreductase. The hit compound **3a** (RTC1) remained the most efficacious with a 2.57 fold increase in glucose uptake compared

to vehicle control and micromolar inhibition of NADH:ubiquinone oxidoreductase (IC_{50} = 27 μM). *In vitro* DMPK and *in vivo* PK studies are also described, where results suggest that **3a** (RTC1) would not be expected to provoke adverse drug-drug interactions, yet be readily metabolised, avoid rapid excretion, with a short half-life, and have good tissue distribution. The overall results indicate that aryl piperazines, and **3a** (RTC1) in particular, have potential as effective agents for the treatment of diabetes.

1. Introduction

Type 2 diabetes mellitus (T2DM) has become one of the great modern public health threats in terms of human, social, and economic costs [1]. T2DM is a chronic condition where blood glucose levels are raised due to the body's inability to produce enough of the hormone insulin or to use insulin effectively. Insulin is an essential hormone produced in the pancreas and plays a critical role in the transport of glucose from the bloodstream into the body's cells. A lack of insulin or a cells insensitivity to insulin leads to elevated blood glucose levels and hyperglycaemia, which if left unchecked can result in organ damage and the development of disabling and life-threatening health complications [1,2].

The numbers suffering from diabetes are immense and are increasing at an alarming rate. In 2017, there were ~ 425 million diabetic sufferers over the age of 20, and this number is conservatively projected to increase to ~629 million in 2045 [1,2]. There are a number of therapeutic options available, some of which can be administered in the early stages of the development of T2DM or as a combination therapy with injectables. There are several classes of hypoglycemic agents found in the clinic, including metformin, sulfonylureas, thiazolidinediones, DPP-4 inhibitors, SGLT2 inhibitors, GLP-1 receptor agonists and insulin, but the control of diabetes remains variable and unsatisfactory [2-4]. The therapeutic regimens, side effects, and possible off-target actions of some of these options are undesirable and can lead to reduced compliance [5-8]. As a consequence, taking into account the projected and rapid growth in the number of those suffering with diabetes, there is an ever-increasing demand for novel anti-diabetic therapies with defined mechanisms of action.

A common objective for many therapies is the management of blood glucose levels and the avoidance of diabetic hyperglycemia. Different strategies can be employed to achieve this including the stimulation of glucose uptake from the bloodstream, reducing gluconeogenesis, and increasing glucose excretion [3,4]. Therapeutics that increase insulin secretion or increase insulin sensitivity will typically result in an increase in glucose uptake and a reduction in diabetic hyperglycemia. Diabetic hyperglycemia has also been reported to aberrantly upregulate complex I activity in the pancreas, which suggest that complex I could be a promising therapeutic target for diabetes [8,9]. We recently reported the piperazine containing compounds RTC1 and RTB70, which act through the inhibition of NADH:ubiquinone oxidoreductase (complex I of the mitochondrial respiratory chain) to stimulate glucose uptake and restore the glucose handling abilities of diabetic mice [10,11]. The reduction in NADH:ubiquinone oxidoreductase activity provoked by these compounds leads to a change in cellular ADP:ATP and AMP:ATP ratios resulting in the activation of AMP-activated protein kinase (AMPK). Once activated, AMPK rapidly restores cellular energy balance by switching off ATP consuming anabolic pathways and by switching on ATP generating catabolic pathways involving increased glucose uptake [12]. Further studies showed that RTC1 augmented the signalling capabilities of insulin in C2C12 cells stimulated with tumour necrosis factor-alpha (TNF-α), thus preventing TNF-α induced insulin resistance through action as an insulin sensitizer [13]. As a result, a systematic analysis of these

compounds was conducted to further explore their ability to stimulate glucose uptake and use as a potential therapeutic strategy for T2DM.

Herein, we report the synthesis and evaluation of derivatives of compound **3a** (RTC1). The study involved the systematic modification of **3a** (RTC1) at five sites, A-E (Figure 1), in order to explore the relationship between chemical structure and biological activity. This approach allowed us to investigate the importance of the aryl substituent (site A), the nature of the aryl group (site B), the piperazine (site C), the length and nature of the alkyl chain (site D), as well as the terminal group (site E) on the ability to stimulate glucose uptake. Compounds were evaluated for their *in vitro* ability to stimulate glucose uptake and, in a number of cases, for the inhibitory activity against NADH:ubiquinone oxidoreductase in permeabilised mitochondria. A pharmacophore was developed based on the effectiveness of the compound library at stimulating glucose uptake, with preliminary drug metabolism and pharmacokinetic (DMPK) studies also conducted with the hit compound **3a** (RTC1).

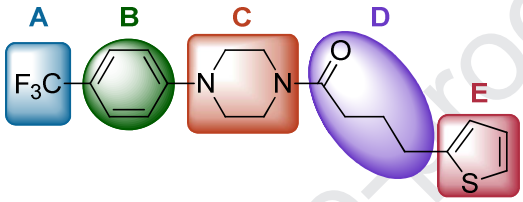
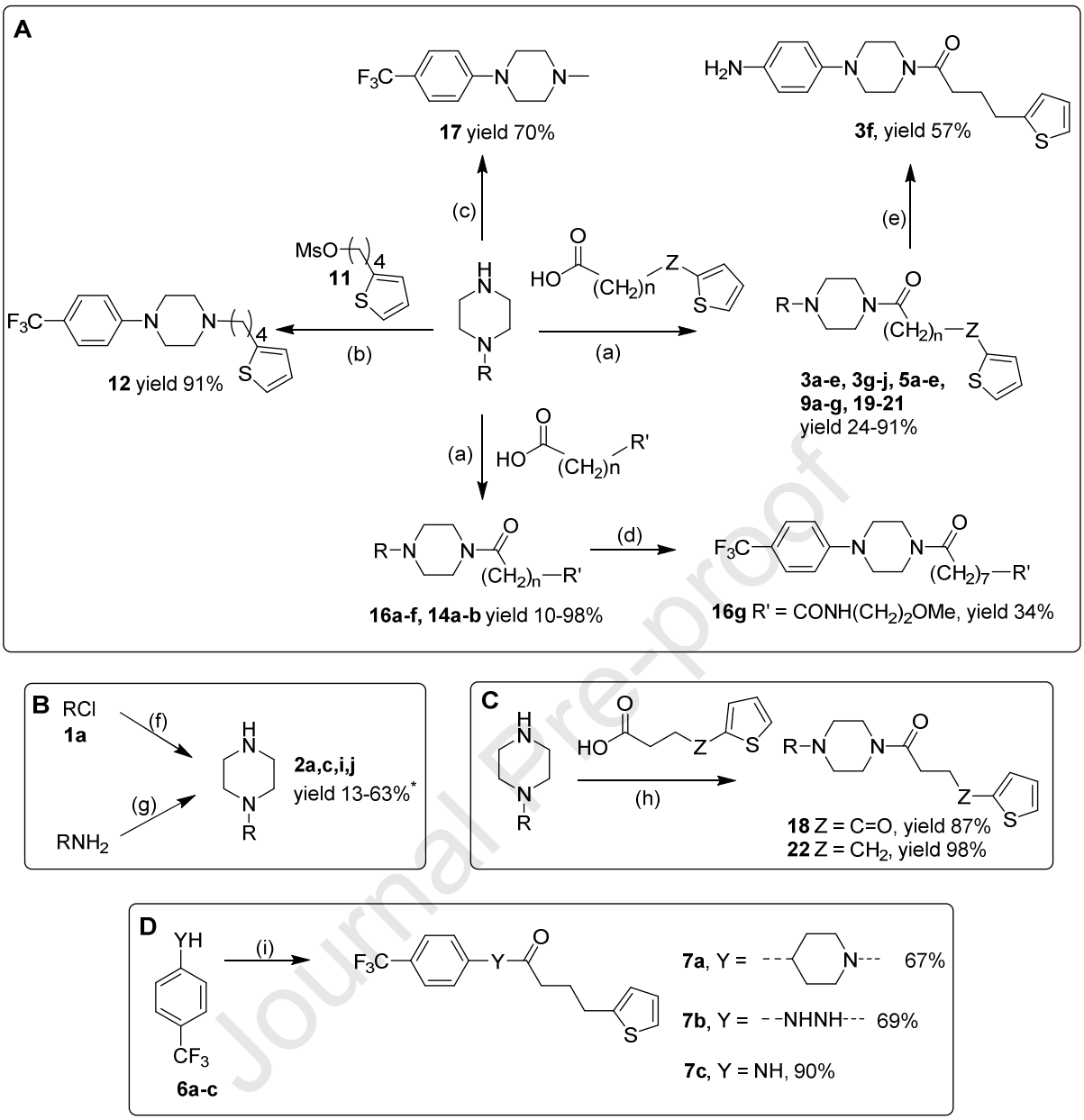


Fig. 1. Chemical structure of **3a** (RTC1) and sites A-E for pharmacomodulation.

2. Results and Discussion

*2.1 Synthesis of hit compound **3a** (RTC1)*

A total of 41 derivatives of the hit compound **3a** (RTC1) were synthesised as shown in Scheme 1. The hit compound **3a** (RTC1) was generated in a two-step process. The intermediate aryl piperazine **2a** was synthesised in 50% yield from 4-chlorobenzotrifluoride **1a** and piperazine by heating to 200 ^{o}C under microwave conditions. Subsequent coupling of **2a** with the commercially available 4-(2-thienyl)butyric acid generated the desired product, **3a** (RTC1), in 86% yield (Scheme 1) [10].



Scheme 1. Synthesis of **3a** (RTC1) derivatives. Reagents and conditions: **A.** (a) HOBt, TBTU, NEt_3, DMF, overnight, rt; (b) Na_2CO_3, MeCN, N_2 atmosphere, 24 h, reflux; (c) EtOH, formic acid, formaldehyde, 3 h, reflux; (d) from compound **16e**, 2-methoxyethanamine, HOBt, TBTU, NEt_3, DMF, 20 h, rt; (e) from compound **3e**, PtO_2, H_2 atmosphere, 16.5 h, rt.
B. (f) Piperazine, NMP, 30 mins, 200 ^oC, MW (**2a**, R = 4-(trifluoromethyl)phenyl, 50% yield; **2i**, R = 2-chloro-4-(trifluoromethyl)phenyl), 63% yield; **2j,** R = 2-bromo-4-(trifluoromethyl)phenyl), 23% yield); (g) (i) bis(2-chloroethyl)amine.HCl, bis(2-methoxyethyl) ether, 16 h, reflux, (ii) 5% NaOH (aq.), 4 h, rt (**2c**, R = *p*-tolyl, 13% yield). *Other amine/piperazine intermediates were commercially available.
C. (h) HOBt, TBTU, NEt_3, DMF, overnight, rt.
D. (i) 4-(2-Thienyl)butyric acid, HOBt, TBTU, NEt_3, DMF, overnight, rt.

2.2 Synthesis of derivatives with variations at site A-E.

The first structural modifications made were those concerned with variations at site A, the substitution pattern of the aryl ring, and were undertaken in order to study the effect and importance of the CF_3 substituent on activity. This involved (i) variation of the *para* substituent on the aryl ring (**3a-3f**); (ii) changing the position of the CF_3 substituent (**3g**), and (iii) bis substitution of the aryl ring (**3h-j**) (Table 1). The key intermediate, the aryl piperazine, was commercially available in some but not all cases. The bis-substituted 1-(2-chloro-4-(trifluoromethyl)phenyl)piperazine **2i** and 1-(2-bromo-4-(trifluoromethyl)phenyl)piperazine **2j** were both synthesised from the corresponding aryl chloride and piperazine via an S_NAr reaction in NMP under microwave conditions (Scheme 1, (a)). The alkyl substituted 1-(p-tolyl)piperazine **2c** could not be generated by this method, and instead was synthesised from the corresponding aniline and bis(2-chloroethyl)amine via a substitution/cyclisation sequence (Scheme 1, (b)). The final step in the synthesis of compounds **3a-j** was the coupling reaction between the aryl piperazine and 4-(2-thienyl)butyric acid, using HOBt and TBTU as coupling reagents (Scheme 1, (c)). This method gave the desired products in yields ranging from 24 to 86% for the final step. Compound **3f** was generated from the *para*-nitro substituted **3e** via the reduction of the nitro group using H_2 over PtO_2 in a 57% yield.

We next explored variations of the aryl ring, site B. To do this, the aryl ring was changed to a substituted pyridine (**5a-c**) as well as cyclic and acyclic alkyl groups (**5d-e**) (Scheme 1, Table 1). In all cases, **5a-e**, the corresponding piperazines were commercially available, which allowed the target compounds to be synthesized in a single coupling reaction with yields ranging from 68-91%.

Changes at site C allowed us to explore the importance of the piperazine ring. This required the synthesis of compounds **7a-c**, where the piperazine ring was replaced with a piperidine ring (**7a**), a hydrazine group (**7b**), and an NH (**7c**) (Scheme1, Table 2). The appropriate starting amine or hydrazine for each target compound were commercially available, with subsequent coupling using 4-(2-thienyl)butyric acid generating **7a-c** in a single step (Scheme 1, 67-90% yield).

Exploration of site D required the synthesis of derivatives with variations in the alkyl chain linking the piperazine motif and the thiophene heterocycle. This included the generation of compounds **9a-e**, where the changes in the alkyl chain length was investigated through sequential addition of single methylene units (Scheme 1, Table 3, 44-82% yield). Compounds **9f-g** were also synthesised, using the same coupling methodology, where additional functional groups were added to the chain including a sulfur atom and a carbonyl group (Scheme 1, Table 3, 63-84% yield). The hit compound **3a** (RTC1) contains an amide carbonyl group adjacent to the piperazine ring. The importance of this amide carbonyl for biological activity was explored though the synthesis and evaluation of compound **12**, which lacks the amide carbonyl (Scheme 1, Table 3**)**. Compound **12** was synthesised via a three step process culminating in a S_N2 type reaction between 1-(4-(trifluoromethyl)phenyl)piperazine and the mesylate ester **11**, 70% yield. Mesylate ester **11** was generated by the reduction of the starting carboxylic acid, followed by mesylation of the resulting primary alcohol **10** (see experimental section).

The importance of a terminal thiophene heterocycle for biological activity, as found in compound **3a** (RTC1), also required investigation. To explore this we removed the thiophene, replacing it with a methyl group **14a** and a pyrazole heterocycle **14b** (Scheme 1, Table 4).

Both **14a** and **14b** were synthesised in a single step from the corresponding carboxylic acid and 1-(4-(trifluoromethyl)phenyl)piperazine in a 57% and 10% yield respectively.

Finally, we undertook an investigation where variations were made at more than one site. This allowed us to explore additional chemical space and the effect that multiple structural variations would have on biological activity. Analogues **16a-f** were synthesised with variations at both sites D and E (Scheme 1, Table 5). Here variations included methyl, furyl, and ester terminal groups as replacements for the thiophene heterocycle in **3a** (RTC1), as well as variations in chain length. All compounds, except **16g**, were synthesised in a single coupling step (Scheme 1, 31-98% yield). Compound **16g** required an additional step where compound **16e** was coupled with 2-methoxyethanamine to give compound **16g** (Table 5). Compound **17** was also generated, where the entire alkyl chain-thiophene ring motif is removed, using formic acid and formaldehyde (Scheme 1, Table 5, 70% yield). Compound **18**, with variations at site C and D, was synthesised in a single step from the commercially available 4-(4-trifluoromethyl)phenyl)piperidine and 4-oxo-4-(2-thienyl)butanoic acid (Scheme 1, Table 5, 87% yield). Compounds **19-21** had variations at both B and D sites and were generated using the same one step process from commercially available starting (Scheme 1, Table 5, 63-71% yield). Compound **22** was synthesised, with variations at both the A and C sites, where the CF_3 substituent of **3a** (RTC1) is removed from the aryl ring and the piperazine saturated heterocycle is replaced by a piperidine (Scheme 1, Table 5, 98% yield). Finally, compound **23** was purchased from a commercial supplier, with compound **24** generated by the straight forward esterification of **23**.

*2.8 In vitro evaluation of glucose uptake by **3a** (RTC1) and its derivatives.*

With the desired compounds in hand, we next undertook the evaluation of their ability to stimulate glucose uptake in C2C12 mouse muscle cells using a tritiated version of deoxy-2-glucose, a derivative of glucose that cannot be metabolized [10, 14]. Results from this assay are presented as fold change, induced with a 10 μM treatment, compared with the vehicle control, DMSO (Tables 1-5). The *in vitro* glucose uptake data revealed that chemical structure had a significant impact on activity. Full experimental details can be found in the experimental section and supporting information.

2.8.1 In vitro glucose uptake for site A modifications.

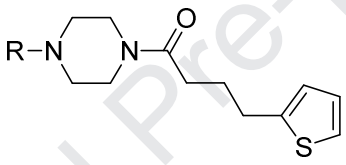
For site A modifications, which entail changes to the substitution of the aryl ring, all results showed a reduction or loss of glucose uptake activity compared to **3a** (RTC1) (Figure 1, Table 1). Removal of the *p*-CF_3 substituent (**3b**), or its replacement with the smaller hydrophobic methyl (**3c**) substituent, resulted in a complete loss of activity. Replacing the CF_3 substituent with a single fluorine atom (**3d**), or another electron withdrawing substituent such as a nitro substituent (**3e**), resulted in complete loss of activity, as did its replacement with the electron donating amino substituent (**3f**). Moving the CF_3 substituent from the *para* to the *ortho* position (**3g**) also resulted in complete loss of activity. The only A type modifications that resulted in active compounds, albeit with reduced activity, was the replacement of the *p*- CF_3 substituent with two *meta* CF_3 substituents (**3h**) or keeping the *p*-CF_3 substituent and adding an *ortho*-Cl substituent (**3i**). From the type A results, it can be suggested that the presence of a CF_3 substituent on the aromatic ring is important for biological activity, and that a single CF_3 substituent at the *para* position is preferable over two CF_3 substituents. CF_3 substituents are well known for the hydrophobic and lipophilic

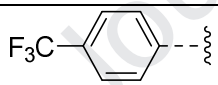
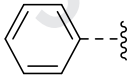
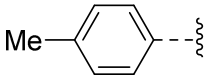
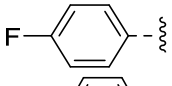
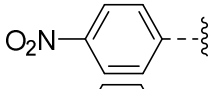
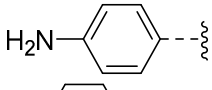
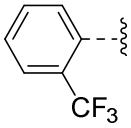
nature, and can aid transport across cell membranes, as well as contribute to binding in hydrophobic enzyme pockets [15,16].

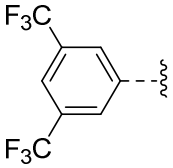
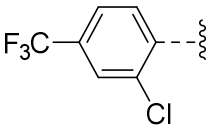
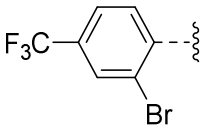
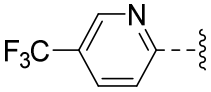
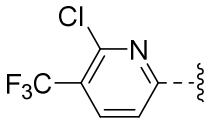
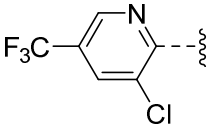
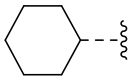
2.8.2 In vitro glucose uptake for site B modifications.

For site B modifications changes were made to the aryl ring itself (Figure 1, Table 1). The employment of a pyridine ring in place of the phenyl ring resulted in compound **5a**, which was inactive in the glucose uptake assay. Compound **5c**, containing a 3-chloro-5-(trifluoromethyl)pyridine in place of the phenyl ring, gave statistically significant activity but at a low level and considerably lower than **3a** (RTC1). Replacing the aromatic ring with an aliphatic cyclohexyl ring (**5d**) did generate an active compound but again with activity levels lower than **3a** (RTC1). Replacement of the aryl ring with a simple aliphatic acyclic group (methyl, **5e**) resulted in complete loss of activity. The results for compounds **5a**-**5e** suggest that the presence of an aryl ring is important for activity and replacement with a pyridine or aliphatic ring has a detrimental effect on activity levels.

Table 1
In vitro glucose uptake activity of compounds with variations at sites A, **3a-j**, and site B, **5a-e**.



Compound	R	Glucose uptake	
		Fold change ± SEM	Active (Y) with p-value or inactive (N)
3a (RTC1)		2.57 ± 0.08	Y (***)
3b		0.96 ± 0.07	N
3c		1.00 ± 0.42	N
3d		1.16 ± 0.09	N
3e		1.06 ± 0.10	N
3f		1.32 ± 0.12	N
3g		0.85 ± 0.02	N

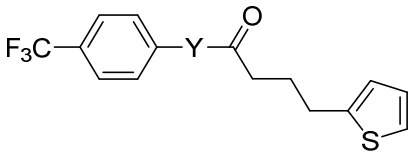
3h		1.42 ± 0.09	Y (*)
3i		1.58 ± 0.15	Y (***)
3j		1.02 ± 0.04	N
5a		1.00 ± 0.09	N
5b		1.22 ± 0.10	N
5c		1.13 ± 0.02	Y (*)
5d		1.29 ± 0.04	Y (***)
5e		0.84 ± 0.03	N

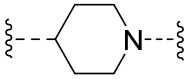
All data are expressed as means ± standard error of the means (SEM) of a number of experiments (n) performed for that compound. Statistical analysis was performed using one-way analysis of variance (ANOVA) using GraphPad Prism 5.0 with a threshold of significance defined as a p-value <0.05 (95% confidence in result) or a one star significance (*), p-value < 0.01 or two stars significance (**), and p-value < 0.0001 or three stars significance (***). Only compounds which received stars significance in the glucose uptake assay were considered "active" compounds. Full experimental details can be found in the experimental section and supporting information.

2.8.3 In vitro glucose uptake for site C modifications.

Three compounds were evaluated with site C modifications (Figure 1, Table 2). Here, replacement of the piperazine ring with a piperidine ring (**7a**) resulted in an active compound with a 2-fold increase in activity compared to the vehicle control. Although a strong result, the piperidine containing **7a** was still less active that **3a** (RTC1), which itself generated a 2.5-fold increase in activity. Surprisingly, replacement of the piperazine ring with a hydrazine motif (**7b)** resulted in an active compound, albeit with a considerably lower level of activity than **3a** (RTC1). The third site C compound evaluated was **7c**, where the piperazine ring was replaced by an NH group, creating an amide linkage between the aryl ring and the rest of the molecule. As one might have expected, this generated a compound that was inactive in the glucose uptake assay. The set of results generated under site C modifications would suggest that the presence of a piperazine ring is optimal for activity, but that replacement with a piperidine ring does not dramatically reduce activity.

Table 2
In vitro glucose uptake activity of compounds **7a-c**.



Compound	Y	Glucose uptake	
		Fold change ± SEM	Active (Y) with p-value or inactive (N)
7a		2.14 ± 0.30	Y (***)
7b		1.24 ± 0.05	Y (**)
7c	NH	1.11 ± 0.08	N

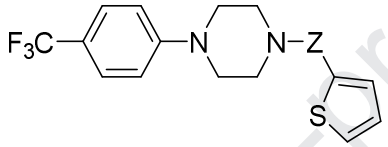
All data are expressed as means ± standard error of the means (SEM) of a number of experiments (n) performed for that compound. Statistical analysis was performed using one-way analysis of variance (ANOVA) using GraphPad Prism 5.0 with a threshold of significance defined as a p-value <0.05 (95% confidence in result) or a one star significance (*), p-value < 0.01 or two stars significance (**), and p-value < 0.0001 or three stars significance (***). Only compounds which received stars significance in the glucose uptake assay were considered "active" compounds. Full experimental details can be found in the experimental section and supporting information.

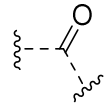
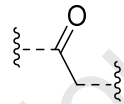
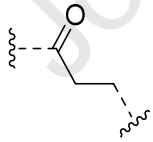
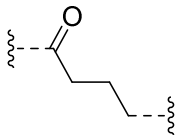
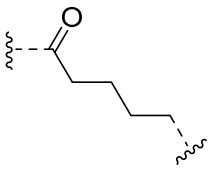
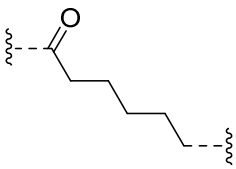
2.8.4 In vitro glucose uptake for site D modifications.

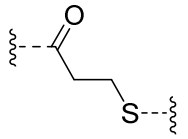
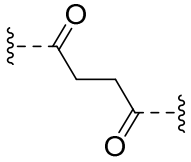
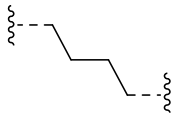
Site D modifications involved the study of the length and nature of the chain connecting the piperazine ring to the thiophene heterocycle. Here, most of the compounds generated could effectively stimulate glucose uptake to some degree, although all were less active than **3a** (RTC1) (Figure 1, Table 3). Compounds **9a**-**9e** explored the length of the methylene chain, with **9a** having no methylene chain and **9e** having a five carbon chain in place. The results for **9a**-**9e** suggest that the alkyl chain in **3a** (RTC1) is important for biological function, as activity was lost when the chain was completely removed in compound **9a**. Activity was also reduced when the alkyl chain was shortened but not fully removed, as was the case for compound **9c** (not active). Lengthening the alkyl chain (**9d** and **9e**) also resulted in a decrease in activity, but not a complete loss in activity. One possible explanation is that a certain alkyl chain length is required for the molecule to bind strongly in an active/binding site. Too short or too long a chain may result in a poor fit and weak binding. Also, an entropy effect could also be considered for the longer chain compounds. The longer chain compounds are able to adopt a larger number of conformations when unbound. As such, upon binding there will be a larger increase in order, entropy cost, and hence the binding of such molecules may be less thermodynamically favourable when compare to the shorter chained **3a** (RTC1) [17,18].

The importance of the carbonyl group in **3a** (RTC1) was explored by generating a derivative (**12**) where this functional group was absent. Compound **12** was considerably less active that **3a** (RTC1), although activity was not completely eliminated suggesting that the presence of the carbonyl group was important for activity but not absolutely essential (Table 3). Compounds **9f** and **9g** all contained modifications to the methylene chain but kept the carbonyl group. These included replacing a methylene unit with a sulfur atom (**9f**) or a carbonyl group (**9g**). All were less active than **3a** (RTC1), although compound **9f** containing the S atom, showed a 2-fold increase in activity compared to vehicle control. This result compared favourably with the 2.57-fold increase observed for **3a** (RTC1).

Table 3
In vitro glucose uptake activity of compounds **3a**, **9a-g**, and **12**.



Compound	Z	Glucose uptake	
		Fold change ± SEM	Active (Y) with p-value or inactive (N)
9a		0.93 ± 0.07	N
9b		1.33 ± 0.04	Y (***)
9c		1.09 ± 0.08	N
3a (RTC1)		2.57 ± 0.08	Y (***)
9d		1.78 ± 0.13	Y (***)
9e		1.33 ± 0.05	Y (***)

9f		2.14 ± 0.23	Y (***)
9g		1.19 ± 0.03	Y (***)
12		1.36 ± 0.09	Y (***)

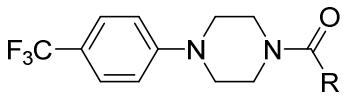
All data are expressed as means ± standard error of the means (SEM) of a number of experiments (n) performed for that compound. Statistical analysis was performed using one-way analysis of variance (ANOVA) using GraphPad Prism 5.0 with a threshold of significance defined as a p-value <0.05 (95% confidence in result) or a one star significance (*), p-value < 0.01 or two stars significance (**), and p-value < 0.0001 or three stars significance (***). Only compounds which received stars significance in the glucose uptake assay were considered "active" compounds. Full experimental details can be found in the experimental section and supporting information.

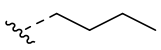
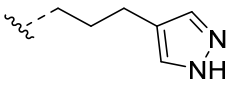
2.8.5 In vitro glucose uptake for (i) site E modifications and (ii) a combination of both site D & site E modifications.

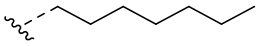
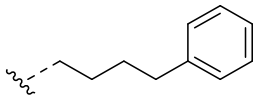
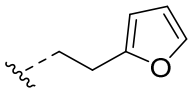
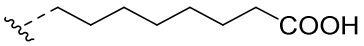
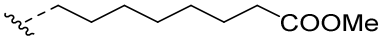
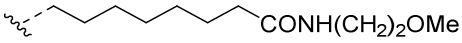
Two compounds were evaluated with site E modifications (**14a** and **14b**) (Figure 1, Table 4). Both compounds were active with statistical significance but were considerably less active than **3a** (RTC1), suggesting that a thiophene ring at site E is not essential for activity but that its replacement results in reduced activity.

The effect of modifying both sites D & E simultaneously was explored with compounds **16a-g** (Figure 1, Table 4). Here, all compounds except **16d** and **16f** were active with statistical significance. However, all compounds were less active that **3a** (RTC1), with **16c** and **16g** proving most active with a 1.95- and 2.35-fold increase, respectively, compared to the vehicle control. This compares favourably to the 2.57-fold increase of **3a** (RTC1). The results in Table 4 suggest that structural modification at the D-E sites of the molecule is better tolerated than those at the A-B sites.

Table 4
In vitro glucose uptake activity of compounds **14a-b** and **16a-g**.



Compound	R	Glucose uptake	
		Fold change ± SEM	Active (Y) with p-value or inactive (N)
14a		1.28 ± 0.11	Y (**)
14b		1.35 ± 0.11	Y (**)
16a		1.43 ± 0.09	Y (***)

16b		1.38 ± 0.05	Y (***)
16c		1.95 ± 0.09	Y (***)
16d		1.11 ± 0.04	N
16e		1.38 ± 0.06	Y (***)
16f		1.08 ± 0.08	N
16g		2.35 ± 0.25	Y (***)

All data are expressed as means ± standard error of the means (SEM) of a number of experiments (n) performed for that compound. Statistical analysis was performed using one-way analysis of variance (ANOVA) using GraphPad Prism 5.0 with a threshold of significance defined as a p-value <0.05 (95% confidence in result) or a one star significance (*), p-value < 0.01 or two stars significance (**), and p-value < 0.0001 or three stars significance (***). Only compounds which received stars significance in the glucose uptake assay were considered "active" compounds. Full experimental details can be found in the experimental section and supporting information.

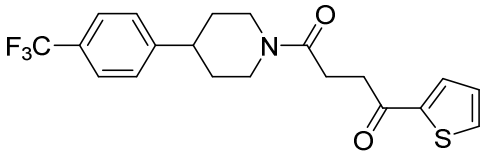
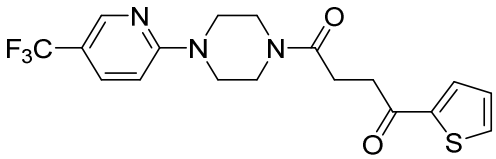
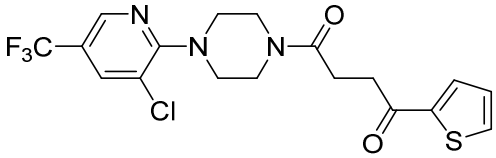
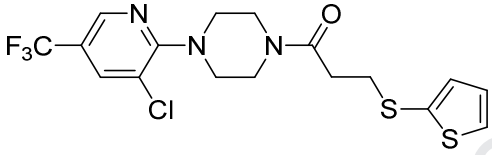
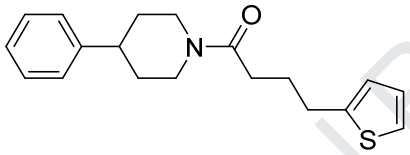
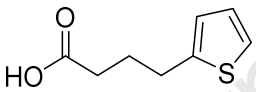
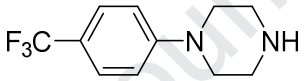
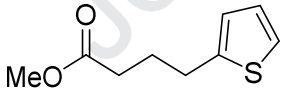
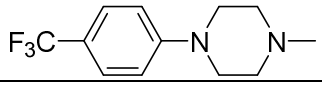
2.8.6 In vitro glucose uptake for modifications at a combination of sites.

A further five compounds (**18**-**22**) with modifications at more than one site were also evaluated for their ability to stimulate glucose uptake *in vitro* (Table 5). As expected, given the effects of the single modifications, these compounds generated lower levels of glucose uptake compared to **3a** (RTC1). Four of the five compounds gave low, but statistically significant levels of activity. Compound **22** perhaps generated the most surprising result, as earlier results for compound **3b**, where the CF_3 substituent was removed, generated an inactive compound. However, the activity of compound **22** was quite low with a 1.25 ± 0.12 fold change compared to vehicle control. Compound **3a** (RTC1) containing both the CF_3 and a piperazine ring, and compound **7a** containing both the CF_3 and the piperidine ring, generated substantially higher levels of activity than compound **22** (2.57 fold change and 2.14 fold change respectively). This would again suggest that the presence of the CF_3 is important for activity.

The starting materials used in the synthesis of **3a** (RTC1), namely compounds **23** and **2a**, along with their methylated forms (**24** and **17**), were also evaluated (Table 5). Both starting materials showed very low levels of activity, with their methylated forms proving to be inactive. This set of results would suggest that neither the thiophone alkyl chain (**23**, **24**), nor the aryl piperazine (**2a**, **17**) alone is sufficient to stimulate glucose uptake, and that a more complex molecule is required. It also suggests that **3a** (RTC1) is not acting as a prodrug, with cleavage/hydrolysis *in vitro* generating the starting materials as the active compounds.

Table 5
In vitro glucose uptake activity of compounds **18**-**22**, **23**, **2a**, **24**, and **17**.

Compound	Structure	Glucose uptake

		Fold change ± SEM	Active (Y) with p-value or inactive (N)
18		1.31 ± 0.05	Y (***)
19		1.22 ± 0.06	N
20		1.26 ± 0.06	Y (*)
21		1.31 ± 0.02	Y (***)
22		1.25 ± 0.12	Y (*)
23		1.16 ± 0.04	Y (*)
2a		1.16 ± 0.06	Y (*)
24		1.09 ± 0.04	N
17		0.92 ± 0.07	N

All data are expressed as means ± standard error of the means (SEM) of a number of experiments (n) performed for that compound. Statistical analysis was performed using one-way analysis of variance (ANOVA) using GraphPad Prism 5.0 with a threshold of significance defined as a p-value <0.05 (95% confidence in result) or a one star significance (*), p-value < 0.01 or two stars significance (**), and p-value < 0.0001 or three stars significance (***). Only compounds which received stars significance in the glucose uptake assay were considered "active" compounds. Full experimental details can be found in the experimental section and supporting information.

2.9 Molecular modelling.

The key features that are responsible for biological function were identified using pharmacophore model generation (Biovia, Discovery Studio). These features consisted of 4 hydrophobics (covering sites A, B, C and E) and a hydrogen bond acceptor (HBA) feature covering site D and 4 exclusion volumes defining the shape of the pocket (Figure 2). One

mis-matched feature was permitted for compound alignment to the pharmacophore. For example, the importance of the carbonyl group in **3a** (RTC1) was explored by analogue **12** which was missing this functional group. While compound **12** was considerably less active that **3a** (RTC1), the activity was not completely eliminated suggesting that the presence of the carbonyl group was important for activity but not absolutely essential. Hence, additional flexibility was incorporated into the pharmacophore model. A distinction is observed between the fit to the pharmacophore of a very active compound such as **3a** (RTC1) (Figure 2), with a 2.57-fold change in the glucose uptake assay (Table 4), and many inactive compounds. Hence, the pharmacophore model could be beneficial in future SAR optimisation of the compounds for the glucose uptake assay.

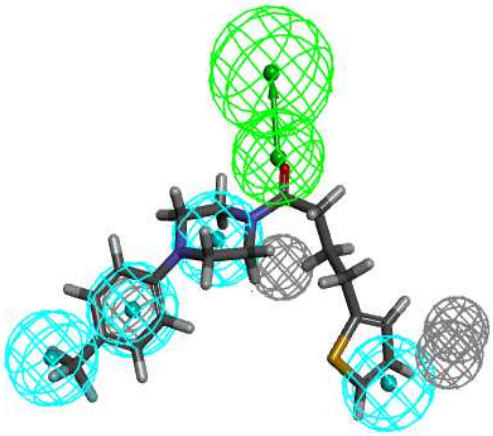


Fig. 2. Pharmacophore model with four hydrophobic features (in cyan), a hydrogen bond acceptor feature (in green), and 4 exclusion volumes (in grey) based on the inactive compounds. Compound **3a** (RTC1), with a 2.57 fold change and a p value of < 0.0001 in the glucose uptake assay, is shown matching the pharmacophore with a fit value of 1.89.

*2.10 In vitro evaluation of NADH:ubiquinone oxidoreductase inhibition by **3a** (RTC1) and its derivatives.*

Both **3a** (RTC1) and **21** (RTB70) have been reported by this group to act through the inhibition of NADH:ubiquinone oxidoreductase (complex I of the mitochondrial respiratory chain), stimulating glucose uptake and restoring the glucose handling abilities of diabetic mice [10]. As such, a number of the most active compounds in this glucose uptake study were evaluated for their inhibitory activity against NADH:ubiquinone oxidoreductase in permeabilised mitochondria (Table 6). For comparison, a number of the inactive compounds were also evaluated in the NADH:ubiquinone oxidoreductase assay (Table 6). All of the active compounds evaluated were also found to be active in the NADH:ubiquinone oxidoreductase assay. The compounds that were most active in the glucose uptake assay were not necessarily the most active in the NADH:ubiquinone oxidoreductase assay (Table 6). This may be due to the difference between a whole cell assay system and an inhibition assay, where for the latter the cell membrane and other cellular machinery have been removed. Furthermore, and to our surprise, all of the inactive compounds evaluated showed some activity in the NADH:ubiquinone oxidoreductase assay (Table 6). One possible explanation is that in the whole cell glucose uptake assay, the compounds could not pass the cell membrane, avoid efflux mechanisms, or survive the cells internal machinery (e.g. hydrolysis, enzyme catalysed degradation) and hence were inactive [19]. In an inhibition assay, such as

the NADH:ubiquinone oxidoreductase assay, these barriers and challenges are removed, and this could allow the compounds to access the complex I active site and inhibit its activity. As such, whole cell assay systems can be useful indicators of *in vivo* biological potency. Hollenback *et al.* have reported a similar trend in their research where they tested substrate specificity in membrane and whole cell assays [20]. Research by Hernandez *et al.* also compared enzyme assays with a whole cell based assay. They concluded that whole cell based assays have the advantage of only selecting compounds that are able to penetrate cells and reach the intracellular targets [21].

Table 6

NADH:ubiquinone oxidoreductase assay results for some of the aryl piperazine compounds.

Compound	IC_{50} (μM)	Glucose uptake fold change ± SEM
3a (RTC1)	27	2.57 ± 0.08
3i	46	1.58 ± 0.15
7a	15	2.14 ± 0.30
9d	60	1.78 ± 0.13
9f	14	2.14 ± 0.23
16c	6	1.95 ± 0.09
3b*	133	0.96 ± 0.07
3e*	17	1.06 ± 0.10
5e*	161	0.84 ± 0.03
7c*	92	1.11 ± 0.08
17*	67	0.92 ± 0.07

* Inactive in glucose uptake assay.

2.11 Predicted physiochemical properties.

In general, the compounds are predicted to have low-to-good solubility, good absorption properties, and high-to-medium predicted blood brain barrier (BBB) penetration (Biovia Discovery Studio). These ADMET screening results are summarized in Tables 1-7 of the supplementary information. For example, compound **3a** (RTC1) is predicted to have a low solubility; good absorption and to readily pass the BBB. It is also likely to bind well to plasma proteins and not to be hepatotoxic.

*2.12 Physiochemical properties, in vitro DMPK, and in vivo PK of **3a** (RTC1)*

A preliminary study was undertaken in order to offer some insight into the physiochemical properties, *in vitro* DMPK, and *in vivo* PK of **3a** (RTC1). The results from this preliminary

study are encouraging, however only tentative conclusions are made due to the limitations of the study. See supporting information for additional experimental detail and supporting data.

Thermodynamic solubility studies revealed the aqueous solubility of **3a** (RTC1) to be 0.004 mg/mL (± 0.001 SEM). The low solubility of **3a** (RTC1) is believed to be due to the lipophilic properties of the compound (logP value of 4.79). However, as removal of the CF_3 moiety decreased the logP value and consequentially the activity of derivatives, this property is believed to be central to the activity of **3a** (RTC1), as it may facilitate lipid membrane permeability and promote target engagement. *In vitro* analysis of P-glycoprotein (P-gp) - mediated efflux, in hMDR1-MDCK seeded filters, demonstrated **3a** (RTC1) to be a highly permeable compound with a passive permeability (P_{app}) value of 27.1×10^{-6} cm/sec (± 1.9 SD), similar to that of the positive control, propranolol (21.8×10^{-6} cm/sec, ± 1.0 SD). Under these conditions **3a** (RTC1) produced an efflux ratio of 0.9, suggesting the compound does not act as a P-gp substrate. The administration of **3a** (RTC1) should therefore not provoke adverse drug-drug interactions [22] as many observed drug-drug interactions are caused by the inhibition of both P-gp and the cytochrome P-450 enzymes (CYP450s).

The effect of **3a** (RTC1) on recombinant human CYP450 isozymes was also investigated and demonstrated weak-moderate inhibition of the major human CYP450s and hence a reduced risk of unwanted drug-drug interactions. CYP1A2 (53.7% inhibition, ± 0.3 SEM), CYP2C9 (74.2% inhibition, ± 3.2 SEM), CYP2C19 (75.5% inhibition, ± 0.5 SEM), CYP2D6 (-2.7% inhibition, ± 4.9 SEM), CYP**3a**4 (80.2% inhibition, ± 1.5 SEM), see supporting information.

Hepatocytes and microsomes were then used to gain an understanding of the way in which **3a** (RTC1) would be metabolised. In both species tested, human and mouse, **3a** (RTC1) (1 µM) demonstrated a shorter half-life and faster intrinsic clearance than the control drug, verapamil, indicating that **3a** (RTC1) would be readily metabolised by the liver *in vivo* (Table 7). In addition to this, **3a** (RTC1) demonstrated no adverse effects on primary rat hepatocytes, nor did it inhibit the hERG channel [10].

Table 7
In vitro assessment of **3a** (RTC1) metabolic stability.

Compound	Species	Microsome		Cryopreserved Hepatocytes		
		$T_{1/2}$ [a] (min)	CL_{int} [b] (µL/min/mg)	$T_{1/2}$ [a] (min)	CL_{int} [c] (µL/min/10^6 cells)	E_H [d]
Verapamil	Human	23	60	633	2	0.16
	Mouse	24	57	288	3	0.29
3a (RTC1)	Human	12	116	3	346	0.98
	Mouse	6	226	125	8	0.48

[a] Half-life. [b] Intrinsic clearance (µL/min/mg). [c] Intrinsic clearance (µL/min/10^6 cells). [d] Hepatic extraction ratio. n = 2, see supporting information.

Compound **3a** (RTC1) exhibited high mouse and human serum protein binding (Table 8), which would prevent the rapid excretion of the compound. Compound **3a** (RTC1) was also observed to be relatively stable in plasma (80 % stable at 2 h), indicative of a compound that would demonstrate good *in vivo* efficacy.

Table 8
Serum protein binding and plasma stability of **3a** (RTC1).

Compound	Species	Serum Protein Binding (% ± SEM)	Plasma Stability $T_{1/2}$ (min)
3a (RTC1)	Human	99.48 ± 0.04	808
	Mouse	99.48 ± 0.01	327

See supporting information.

As with the recent pharmacodynamic study [10], **3a** (RTC1) (10 mg/kg) administered intravenously (IV) or orally to male C57BL/6 mice produced no observable toxic effects. Following IV administration, **3a** (RTC1) had a short half-life of 0.45 h and high plasma clearance. The compound distributed at high levels into all of the tissues analysed apart from muscle, following a similar profile to plasma, with moderate to high clearance. Compound **3a** (RTC1) was also rapidly absorbed following oral dosing, with a similar half-life to post IV administration at 0.76 h. The tissue levels also show a similar distribution profile to the plasma profile, as was seen post IV dosing. Plasma clearance of **3a** (RTC1) was in line with that of mouse liver blood flow (5400 mL/h/kg), indicating metabolism to be the main route of clearance (Table 9). While **3a** (RTC1) exhibited high mouse and human serum protein binding, it had moderate bioavailability (21.4%) suggesting the bound portion of **3a** (RTC1) may act as a reservoir or depot from which **3a** (RTC1) is slowly released as the unbound free form.

Table 9
Pharmacokinetic properties of **3a** (RTC1) *in vivo.*

Parameter	Plasma		Brain		Kidney		Spleen		Liver		Muscle	
	Oral[a]	IV[b]	Oral[a]	IV[b]	Oral[a]	IV[b]	Oral[a]	IV[b]	Oral[a]	IV[b]	Oral[a]	IV[b]
$T_{1/2}$ (h)	0.76	0.45	0.46	0.5	0.57	0.76	1.1	1.1	1	1.3	c.n.c	0.52
T_{max} (h)	0.25	0.03	0.25	0.03	0.25	0.03	0.25	0.03	0.25	0.03	c.n.c	0.03
C_{max} (ng/mL)	787	996	1430	1029	1637	552	1103	587	4875	1833	c.n.c	165
AUC_{last} (h*ng/mL)	520	240	763	246	1224	320	1262	463	4048	487	c.n.c	77.3
AUC_{all} (h*ng/mL)	520	240	763	246	1224	320	1262	463	4048	487	c.n.c	77.3
AUC_{inf} (h*ng/mL)	523	244	774	253	1275	348	1274	475	4089	531	c.n.c	107
Cl (mL/h/kg)		4102		3958		2871		2105		1884		9312
Vd (mL/kg)		1686		1826		3057		3188		3059		7134

[a] Oral and [b] IV solutions were formulated in 10% DMSO, 10% cremaphor, 80% saline.
c.n.c = could not calculate.
Concentrations of **3a** (RTC1) (mean ± SEM) in mouse (C57 Black, ♂) were determined following PO or IV administration at 10 mg/kg (n= 3). See supporting information.

2.13 Statistical analysis

Data presented herein are expressed as means ± standard error of the means (SEM) of a number of experiments (n) performed for that compound. Where possible, statistical analysis was performed using one-way analysis of variance (ANOVA) using GraphPad Prism 5.0 with a threshold of significance defined as a p-value <0.05 (95% confidence in result) or a one star significance (*), p-value < 0.01 or two stars significance (**), and p-value < 0.0001 or three stars significance (***). Only compounds which received stars significance in the glucose uptake assay were considered "active" compounds.

3. Conclusions

To summarise, the synthesis of 41 derivatives of hit compound **3a** (RTC1) and a systematic structure-activity-relationship study are reported where a number of compounds were shown to effectively stimulate glucose uptake *in vitro* and inhibit NADH:ubiquinone oxidoreductase. The key structural features responsible for biological function were also identified via the generation of a pharmacophore model. The hit compound **3a** (RTC1) remained the most effective compound of those evaluated, with a 2.57 fold increase in glucose uptake compared to vehicle control and micromolar inhibition of NADH:ubiquinone oxidoreductase (IC_{50} = 27 µM). The presence of a *p*-CF_3 aryl piperazine motif is important for biological activity, with greater tolerance observed for changes to the alkyl chain and thiophene heterocycle. Preliminary *in vitro* DMPK and *in vivo* PK studies are also described, where encouraging results suggest that **3a** (RTC1) does not act as a P-gp substrate and should not provoke adverse drug-drug interactions. An initial i*n vitro* metabolic stability study, using hepatocytes and microsomes, suggested that **3a** (RTC1) would be readily metabolised by the liver. Intravenous (IV) or oral administration of **3a** (RTC1) to male C57BL/6 mice produced no observable toxic effects in our preliminary study. Following IV administration, **3a** (RTC1) had a short half-life of 0.45 h, showed high plasma clearance, and was distributed at high levels into all of the tissues analysed apart from muscle. While **3a** (RTC1) exhibited high mouse and human serum protein binding, it had moderate bioavailability (21.4 %) suggesting the bound portion of **3a** (RTC1) may act as a reservoir or depot from which **3a** (RTC1) is slowly released as the unbound free form. The overall results suggest that aryl piperazines, and **3a** (RTC1) in particular, have potential as efficacious agents for the treatment of diabetes.

4. Experimental section

4.1 Chemistry

4.1.1. General information

NMR spectra were recorded using Bruker Ascend 500 spectrometer at 293 K operating at 500 MHz for the ^{1}H nucleus and 126 MHz for the ^{13}C nucleus or a Bruker Advance 300 spectrometer operating at 300 MHz for ^{1}H nucleus and 75 MHz for ^{13}C nucleus. All chemical shifts were referenced relative to the relevant deuterated solvent residual peaks or TMS. Chemical shifts were given in ppm downfield from the internal standard and coupling constants were given in Hz. ^{13}C NMR spectra were recorded with complete proton decoupling. Microwave reactions were carried out using a CEM Discover Microwave

Synthesizer with a vertically focused floor mounted infrared temperature sensor, external to the microwave tube. The 10 mL reaction vessels used were supplied from CEM and were made of borosilicate glass. Melting point analyses were carried out using a Stewart Scientific SMP11 melting point apparatus and are uncorrected. High-resolution mass spectrometry (HRMS) was performed in Maynooth University (MU) and the University of Bath (UoB). HRMS in MU was conducted on an Agilent-LC 1200 Series coupled to a 6210 or 6530 Agilent Time-Of-Flight (TOF) mass spectrometer equipped with an electrospray source in both positive and negative (ESI+/−) modes. In UoB, HPLC-ESI-TOF analysis was conducted using an electrospray time-of-flight (MicrOTOF) mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany), which was coupled to an Agilent HPLC stack (Agilent, Santa Clara, CA, United States) consisting of Agilent G1312A binary pump with G1329A autosampler and G1316A column oven. Analyses were performed in ESI positive and negative mode. Data processing was performed using the Compass Data Analysis software scripts (Bruker Daltonik GmbH, Bremen, Germany). Infrared spectra were recorded on a Perkin Elmer Spectrum 100 FT-IR spectrophotometer using a smart endurance single bounce diamond, attenuated total reflection (ATR) cell or potassium bromide (KBr) disks. Spectra were recorded in the region of 4000–600 cm^{-1} and were obtained by the co-addition of 4 scans with a resolution of 4 cm^{-1}. Scintillation counts were obtained using a Wallac MicroBeta scintillation counter (Perkin Elmer). Reactions were monitored with thin layer chromatography (TLC) on Merck Silica Gel F254 plates. Developed sheets were visualised using a portable UVltec CV-006 lamp (λ = 254, 365 nm). Flash column chromatography was performed using Merck Silica gel 60.

*4.1.2 1-(4-(Trifluoromethyl)phenyl)piperazine (**2a**).*

1-Chloro-4-(trifluoromethyl)benzene (1.59 mL, 11.91 mmol) and piperazine (2.00 g, 23.82 mmol) were dissolved in NMP (5 mL) and heated at 200 º C for 30 mins in a microwave reactor. The reaction mixture was purified using column chromatography (1:9, MeOH:DCM) to give a white solid, 1.42 g (50%). Rf: 0.2 (9:1, DCM:MeOH); IR (ATR): υ (cm^{-1}) 3257, 2836, 1668, 1613, 1323, 1101, 1067; ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.48 (d, *J* = 8.8 Hz, 2H), 6.92 (d, *J* = 8.8 Hz, 2H), 3.28 – 3.21 (m, 4H), 3.07 – 3.00 (m, 4H), 2.01 (bs, 1H); ^{1}H NMR data matches literature data [31]; ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 153.7, 126.4 (q, *J* = 3.8 Hz), 124.7 (q, *J* = 270.9 Hz), 120.6 (q, *J* = 32.6 Hz), 114.6, 49.1, 45.9; ^{13}C NMR data matches literature data [32]; HRMS calcd for $C_{11}H_{14}F_3N_2$ $[M + H]^+$ 231.1104 found 231.1106.

*4.1.3 1-(p-Tolyl)piperazine (**2c**).*

p-Toluidine (1.00 g, 9.34 mmol) and bis(2-chloroethyl)amine.HCl (1.66 g, 9.34 mmol) were dissolved in bis(2-methoxyethyl)ether (20 mL) and heated at reflux for 16 hrs after which the reaction mixture was allowed to cool to rt. Et_2O was added until the precipitation of a brown solid was complete. The precipitate was collected *via* vacuum filtration and washed with Et_2O (3 x 20 mL) to give the HCl salt of compound **2c**, which was then dissolved in a 5% aqueous NaOH solution (10 mL) and stirred at rt for 4 hrs. The aqueous layer was extracted with DCM (10 mL, followed be 3 x 10 mL) and and the combined organic layers were dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue was purified using flash chromatography to give a brown solid, 221 mg (13%). R_f: 0.2 (1:9, MeOH:DCM); ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.07 (d, *J* = 8.4 Hz, 2H), 6.83 (d, *J* =

8.4 Hz, 2H), 3.14-3.13 (m, 4H), 3.08-3.05 (m, 4H), 2.26 (s, 3H); ^{1}H NMR data matches literature data [33]; HRMS calcd for $C_{11}H_{17}N_2$ $[M + H]^+$ 177.1385 found 177.1394.

*4.1.4 1-(2-Chloro-4-(trifluoromethyl)phenyl)piperazine (**2i**).*

3-Chloro-4-fluorobenzotrifluoride (100 µL, 0.75 mmol) and piperazine (129 mg, 1.5 mmol) were dissolved in NMP (3 mL) and heated at 200 º C for 30 mins in a microwave reactor. The reaction mixture was purified using column chromatography (1:4, MeOH:DCM) to give an orange oil, 124 mg (63%). R_f: 0.2 (1:9, MeOH:DCM); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.61 (d, *J* = 1.4 Hz, 1H), 7.47 (dd, *J* = 8.4, 1.4 Hz, 1H), 7.08 (d, *J* = 8.4 Hz, 1H), 3.10 (s, 8H), 2.78 (bs, 1H); ^{1}H NMR data matches literature data [34]; ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 152.5, 128.6, 127.9 (q, *J* = 3.8 Hz), 125.3 (q, *J* = 33.3 Hz), 124.7 (q, *J* = 3.9 Hz), 123.6 (q, *J* = 272.2 Hz), 120.2, 51.9, 51.4, 50.9, 45.9; HRMS calcd for $C_{11}H_{13}ClF_3N_2$ $[M + H]^+$ 264.06; found 265.0711.

*4.1.5 1-(2-Bromo-4-(trifluoromethyl)phenyl)piperazine (**2j**).*

3-Bromo-4-chlorobenzotrifluoride (200 µL, 1.33 mmol) and piperazine (229 mg, 2.66 mmol) were dissolved in NMP (2 mL) and heated at 200 º C for 30 mins in a microwave reactor. Compound **4** was used without further purification. Clear oil, 95 mg (23%, crude yield). R_f: 0.2 (1:9, MeOH:DCM); ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.46 (d, *J* = 7.8 Hz, 1H), 7.25 – 7.20 (m, 2H), 3.10 (m, 8H), 2.95 (bs, 1H); ^{1}H NMR data matches literature data [35].

4.1.6 Method 1: General procedure for amide bond formation.

Carboxylic acid, HOBt, TBTU, anhydrous NEt_3 and anhydrous DMF were placed in an oven-dried Schlenk tube under a N_2 atmosphere. The resulting solution was stirred at room temperature for 15 mins. A second Schlenk tube was prepared containing amine and anhydrous DMF under a N_2 atmosphere. The resulting solution was transferred, *via* a cannula, to the first Schlenk tube containing the carboxylic acid. The solution was stirred under N_2 overnight. DMF was removed under reduced pressure and the resulting oil was acidified (pH = 3) using a 0.1 M aqueous HCl solution. The aqueous mixture was extracted with DCM (20 mL, followed by 4 x 10 mL). The organic combined layers were washed with a saturated aqueous solution of Na_2CO_3 (3 x 20 mL) and brine (3 x 20 mL) and dried over $MgSO_4$ and the residue was purified using column chromatography.

4.1.7 Method 2: General procedure for amide bond formation.

Carboxylic acid, HOBt, TBTU, NEt_3 and DMF were placed in a round-bottom flask and stirred at room temperature for 15 mins. A second round-bottom flask was prepared containing amine and DMF. The resulting solution was transferred to the first round-bottom flask containing the carboxylic acid. The solution was stirred overnight. DMF was removed under reduced pressure and the residue was purified using column chromatography.

*4.1.8 4-(Thiophen-2-yl)-1-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)butan-1-one (**3a**).*

Prepared from 1-(4-(trifluoromethyl)phenyl)piperazine **2a** (100 mg, 0.43 mmol) and 4-(2-thienyl)butyric acid (50 µL, 0.34 mmol), using HOBt (58 mg, 0.43 mmol), TBTU (140 mg, 0.43 mmol), NEt_3 (100 µL, 0.69 mmol) and DMF (3 mL) and following the general procedure described in method 2 section 4.1.7 [10]. The reaction mixture was stirred at room temperature for 21 h. DMF was removed under reduced pressure and the residue was purified using column chromatography (3:2 EtOAc:*n*-hexane) to give a white solid, 112 mg (86%). R_f: 0.5 (3:2, EtOAc:*n*-hexane); IR (ATR): υ (cm^{-1}) 2925, 2853, 1652, 1611, 1328, 1068; ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.50 (d, *J* = 8.5 Hz, 2H), 7.13 (d, *J* = 4.9 Hz, 1H), 6.94 – 6.89 (m, 3H), 6.81 (bs, 1H), 3.77 (m 2H), 3.56 (m 2H), 3.31 – 3.17 (m, 4H), 2.93 (t, *J* = 7.2 Hz, 2H), 2.40 (t, *J* = 7.4 Hz, 2H), 2.13 – 2.0 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 171.1, 152.9, 144.4, 126.9, 126.6 (q, *J* = 3.7 Hz), 125.6 (q, *J* = 271.3 Hz), 124.6, 123.3, 121.2 (q, *J* = 32.8 Hz), 115.0, 48.3, 48.1, 45.0, 41.1, 32.0, 29.3, 27.0; HRMS calcd for $C_{19}H_{21}F_3N_2OSNa$ $[M + H]^+$ 405.1219 found 405.1228. Matches literature data. [10, 35]

*4.1.9 1-(4-Phenylpiperazin-1-yl)-4-(thiophen-2-yl)butan-1-one (**3b**).*

Prepared from 1-phenylpiperazine (100 µL, 0.64 mmol) and 4-(2-thienyl)butyric acid (84 µL, 0.58 mmol) using HOBt (86 mg, 0.64 mmol), TBTU (205 mg, 0.64 mmol), NEt_3 (129 µL, 0.93 mmol) and DMF (5 mL) and following the general procedure described in method 2 section 4.1.7. The reaction mixture was stirred at room temperature for 16.5 h. DMF was removed under reduced pressure and the residue was purified using column chromatography (3:2, EtOAc:*n*-hexane) to give a dark red oil, 142 mg (78%). R_f: 0.65 (3:2, EtOAc:*n*-hexane); IR (ATR): υ (cm^{-1}) 2915, 1639, 1597, 1227 (C-N); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.30 – 7.23 (m, 2H), 7.11 (d, *J* = 5.1 Hz, 1H), 6.95 – 6.85 (m, 4H), 6.83 – 6.77 (m, 1H), 3.81 – 3.70 (m, 2H), 3.59 – 3.48 (m, 2H), 3.16 – 3.08 (m, 4H), 2.92 (t, *J* = 7.3 Hz, 2H), 2.43 (t, *J* = 7.3 Hz, 2H), 2.05 (p, *J* = 7.3 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 171.0, 151.0, 144.4, 129.2, 126.8, 124.5, 123.2, 120.5, 116.6, 49.7, 49.4, 45.4, 41.5, 32.0, 29.3, 27.0; HRMS calcd for $C_{18}H_{23}N_2OS$ $[M + H]^+$ 315.1526 found 315.1531.

*4.1.10 4-(Thiophen-2-yl)-1-(4-(p-tolyl)piperazin-1-yl)butan-1-one (**3c**).*

Prepared from 1-(*p*-tolyl)piperazine **2c** (221 mg, 1.25 mmol) and 4-(2-thienyl)butyric acid (166 µL, 1.13 mmol) using HOBt (168 mg, 1.25 mmol), TBTU (401 mg, 1.25 mmol), anhydrous NEt_3 (253 µL, 1.82 mmol), and anhydrous DMF (5mL) and following the general procedure described in method 1 section 4.1.6 The reaction mixture was stirred overnight at room temperature under a N_2 atmosphere. Purified using flash chromatography (1:1 EtOAc:*n*-hexane) to give an orange oil, 102 mg (24%). R_f: 0.3 (1:1, EtOAc:*n*-hexane); IR (neat): υ (cm^{-1}) 2917, 1642, 1439, 1232. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.12 (dd, *J* = 5.2, 1.2 Hz, 1H), 7.09 (d, 2H, *J* = 8.6 Hz), 6.92 (dd, *J* = 5.2, 3.4 Hz, 1H), 6.83 (d, *J* = 8.6 Hz, 2H), 6.81-6.80 (m, 1H), 3.78-3.76 (m, 2H), 3.56-3.54 (m, 2H), 3.09-3.07 (m, 4H), 2.92 (t, *J* = 7.3 Hz, 2H), 2.40 (t, *J* = 7.3 Hz, 2H), 2.28 (s, 3H), 2.08-2.02 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 171.0, 148.8, 144.4, 130.2, 129.7, 126.8, 124.5, 123.2, 117.0, 50.3, 50.0, 45.5, 41.5, 32.0, 29.3, 27.0, 20.4. HRMS calcd for $C_{19}H_{25}N_2OS$ $[M + H]^+$ 329.1682 found 329.1687.

*4.1.11 1-(4-(4-Fluorophenyl)piperazin-1-yl)-4-(thiophen-2-yl)butan-1-one (**3d**).*

Prepared from 1-(4-fluorophenyl)piperazine (100 mg, 0.55 mmol) and 4-(2-thienyl)butyric acid (73 µL, 0.50 mmol) using HOBt (74 mg, 0.55 mmol), TBTU (177 mg, 0.55 mmol), anhydrous NEt_3 (123 µL, 0.80 mmol) and anhydrous DMF (5 mL) and following the general procedure described in method 1 section 4.1.6. The reaction mixture was stirred at room temperature for 17 h under a N_2 atmosphere. DMF was removed under reduced pressure and the residue was purified using column chromatography (3:2, EtOAc:*n*-hexane) to give an off-white solid , 119 mg (71%). Mp: 84-86 °C; R_f: 0.6 (3:2, EtOAc:n-hexane); IR (KBr): υ (cm^{-1}) 2824, 1651, 1511, 1437, 1334, 1203; ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.12 – 7.10 (m, 1H), 7.03 – 6.76 (m, 6H), 3.78 – 3.74 (m, 2H), 3.56 – 3.50 (m, 2H), 3.05 – 3.02 (m, 4H), 2.92 (t, *J* = 7.2 Hz, 2H), 2.40 (t, *J* = 7.2 Hz, 2H), 2.09 – 1.99 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 171.0, 157.6 (d, *J* = 238.3 Hz), 147.6 (d, *J* = 2.3 Hz), 144.4, 126.8, 124.5, 123.2, 118.5 (d, *J* = 7.6 Hz), 115.7 (d, *J* = 22.0 Hz), 50.8, 50.4, 45.5, 41.5, 32.0, 29.3, 27.0; Anal. calcd for $C_{18}H_{21}FN_2OS$ C, 54.74; H, 4.84; N, 6.72; found C, 54.73; H, 4.60; N, 6.58; HRMS calcd for $C_{18}H_{22}FN_2OS$ $[M + H]^+$ 333.1431 found 333.1431.

*4.1.12 1-(4-(4-Nitrophenyl)piperazin-1-yl)-4-(thiophen-2-yl)butan-1-one (**3e**).*

Prepared from 1-(4-nitrophenyl)piperazine (150 mg, 0.72 mmol) and 4-(2-thienyl)butyric acid (95 µL, 0.66 mmol) using HOBt (97 mg, 0.72 mmol), TBTU (232 mg, 0.72 mmol), NEt_3 (146 µL, 1.05 mmol), and DMF (6 mL) and following the general procedure described in method 2 section 4.1.7. The reaction mixture was stirred at room temperature for 22.5 h. DMF was removed under reduced pressure and the residue was purified using column chromatography (7:3, EtOAc:DCM) to give an orange solid, 199 mg (84%). Mp: 120-121 °C; R_f: 0.65 (7:3, EtOAc:DCM); IR (ATR): υ (cm^{-1}) 2857, 1648, 1599, 1321, 1163; ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 8.14 (dd, *J* = 9.1, 1.4 Hz, 2H), 7.13 (dd, *J* = 5.1, 1.1 Hz, 1H), 6.95 – 6.90 (m, 1H), 6.86 – 6.78 (m, 3H), 3.84 – 3.76 (m, 2H), 3.65 – 3.56 (m, 2H), 3.47 – 3.39 (m, 4H), 2.93 (t, *J* = 7.2 Hz, 2H), 2.40 (t, *J* = 7.2 Hz, 2H), 2.10 – 2.03 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ (ppm) 171.2, 154.4, 144.3, 139.1, 126.9, 126.0, 124.6, 123.3, 112.9, 47.0, 46.9, 44.7, 40.8, 31.9, 29.2, 26.8; HRMS calcd for $C_{18}H_{21}N_3O_3SNa$ $[M + Na]^+$ 382.1196 found 382.1212.

*4.1.13 1-(4-(4-Aminophenyl)piperazin-1-yl)-4-(thiophen-2-yl)butan-1-one (**3f**).*

Prepared from 1-(4-(4-nitrophenyl)piperazin-1-yl)-4-(thiophen-2-yl)butan-1-one **3e** (143 mg, 0.39 mmol) dissolved in MeOH (10 mL) in a round bottom flask with PtO_2 (20 mg, 0.08 mmol). The resulting mixture was degassed and purged with H_2 (x3), stirred vigorously at room temperature for 16.5 h under an atmosphere of H_2 gas and monitored by TLC (7:1, EtOAc:DCM). The reaction mixture was passed through a bed of Celite and the filtrate was concentrated under reduced pressure. The residue was purified using column chromatography (7:1, EtOAc:DCM) to give a brown oil, 75 mg (57%). R_f: 0.4 (7:1, EtOAc:DCM); IR (ATR): υ (cm^{-1}) 3337, 2815, 1638, 1510, 1223; ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.12 (dd, *J* = 5.1, 1.2 Hz, 1H), 6.92 (dd, *J* = 5.1, 3.4 Hz, 1H), 6.82 – 6.77 (m, 3H, H2), 6.67 – 6.63 (m, 2H), 3.77 – 3.74 (m, 2H), 3.56 – 3.52 (m, 2H), 2.99 – 2.90 (m, 6H), 2.42 – 2.38 (m, 2H), 2.08

– 2.01 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 171.0, 144.5, 144.0, 140.9, 126.8, 124.5, 123.2, 119.3, 116.2, 51.6, 51.2, 45.7, 41.7, 32.1, 29.3, 27.1; HRMS calcd for $C_{18}H_{24}N_3OS$ $[M + H]^+$ 331.1664 found: 331.1669.

*4.1.14 4-(Thiophen-2-yl)-1-(4-(2-(trifluoromethyl)phenyl)piperazin-1-yl)butan-1-one (**3g**).*

Prepared from 1-(2-(trifluoromethyl)phenyl)piperazine (83 µL, 0.43 mmol) and 4-(2-thienyl)butyric acid (57 µL, 0.39 mmol) using HOBt (58 mg, 0.43 mmol), TBTU (139 mg, 0.43 mmol), NEt_3 (60 µL, 0.62 mmol) and DMF (4 mL) and following the general procedure described in method 2 section 4.1.7. The reaction mixture was stirred at room temperature for 22 h. DMF was removed under reduced pressure and the residue was purified using column chromatography (3:2, EtOAc:*n*-hexane) to give a colourless oil, 122 mg (82%). R_f: 0.74 (3:2, EtOAc:*n*-hexane); IR (ATR): υ (cm^{-1}) 2916, 1642, 1312, 1107 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.64 (d, *J* = 7.9 Hz, 1H), 7.56 – 7.48 (m, 1H), 7.33 – 7.21 (m, 2H), 7.12 (dd, *J* = 5.1, 1.1 Hz, 1H), 6.92 (dd, *J* = 5.1, 3.4 Hz, 1H), 6.82 (dd, *J* = 3.3, 0.9 Hz, 1H), 3.76 (bs, 2H), 3.59 – 3.48 (m, 2H), 2.97 – 2.82 (m, 6H), 2.41 (t, *J* = 7.5 Hz, 2H), 2.06 (p, *J* = 7.4 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 171.0, 151.7 (d, *J* = 1.1 Hz), 144.4, 132.8, 127.4 (q, *J* = 28.7 Hz), 127.2 (q, *J* = 5.4 Hz), 126.7, 125.3, 124.4, 124.0, 123.9 (q, *J* = 273.7 Hz), 123.1, 53.7, 53.0, 46.0, 42.0, 32.1, 29.3, 27.0; HRMS calcd for $C_{19}H_{22}F_3N_2OS$ $[M + H]^+$, 383.1399 found 383.1406.

*4.1.15 1-(4-(3,5-bis(Trifluoromethyl)phenyl)piperazin-1-yl)-4-(thiophen-2-yl)butan-1-one (**3h**).*

Prepared from 1-(3,5-bis(trifluoromethyl)phenyl)piperazine (194 mg, 0.65 mmol) and 4-(2-thienyl)butyric acid (85 µL, 0.58 mmol) using HOBt (87 mg, 0.65 mmol), TBTU (207 mg, 0.65 mmol), NEt_3 (129 µL, 0.92 mmol) and DMF (5 mL) and following the general procedure described in method 2 section 4.1.7. The reaction mixture was stirred at room temperature for 14.5 h. DMF was removed under reduced pressure and the residue was purified using column chromatography (3:2, EtOAc:*n*-hexane) to give a clear oil, 223 mg (86%). Mp: 46-49 °C; R_f: 0.78 (3:2, EtOAc:*n*-hexane); IR (ATR): υ (cm^{-1}) 2835, 1655, 1273, 1120; ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.33 (s, 1H), 7.24 (s, 2H), 7.12 (dd, *J* = 5.0 Hz), 6.96 – 6.89 (m, 1H), 6.81 (s, 1H), 3.86 – 3.76 (m, 2H), 3.65 – 3.55 (m, 2H), 3.34 – 3.21 (m, 4H), 2.93 (t, *J* = 7.2 Hz, 2H), 2.41 (t, *J* = 7.2 Hz, 2H), 2.11 – 2.02 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 171.1, 151.3, 144.3, 132.5 (q, *J* = 32.8 Hz), 126.8, 124.6, 123.5 (q, *J* = 273.3 Hz), 123.2, 115.1 (m), 112.7 (m), 48.4, 48.3, 44.9, 41.0, 31.9, 29.2, 26.9; Anal. calcd for $C_{20}H_{20}F_6N_2OS$ C, 53.33; H, 4.48; N, 6.22; found C, 53.41; H, 4.25; N, 6.27; HRMS calcd for $C_{20}H_{20}F_6N_2OSNa$ $[M + Na]^+$ 473.1093 found 473.1107.

*4.1.16 1-(4-(2-Chloro-4-(trifluoromethyl)phenyl)piperazin-1-yl)-4-(thiophen-2-yl)butan-1-one (**3i**).*[35]

Prepared from 1-(2-chloro-4-(trifluoromethyl)phenyl)piperazine **2i** (90 mg, 0.34 mmol) and 4-(2-thienyl)butyric acid (46 µL, 0.32 mmol) using HOBt (47 mg, 0.34 mmol), TBTU (111 mg, 0.34 mmol), NEt_3 (47 µL, 0.51 mmol) and DMF (3 mL) and following the general procedure described in method 2 section 4.1.7. The reaction mixture was stirred at room

temperature for 19 h. DMF was removed under reduced pressure and the residue was purified using column chromatography (3:2, EtOAc:Pet. ether (40-60 ^{o}C)) to give an oil, 111 mg (83%). Mp: 84-86 °C; R_f: 0.7 (3:2, EtOAc:Pet. ether (40-60 ^{o}C)); IR (ATR): υ (cm^{-1}) 2823, 1624, 1325, 1111, 694; ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.65 – 7.62 (m, 1H), 7.48 (dd, *J* = 8.4, 1.7 Hz, 1H), 7.13 (dd, *J* = 5.1, 1.0 Hz, 1H), 7.05 (d, *J* = 8.4 Hz, 1H), 6.95 – 6.91 (m, 1H), 6.84 – 6.79 (m, 1H), 3.86 – 3.76 (m, 2H), 3.64 – 3.56 (m, 2H), 3.09 – 3.02 (m, 4H), 2.93 (t, *J* = 7.3 Hz, 2H), 2.42 (t, *J* = 7.5 Hz, 2H), 2.06 (p, *J* = 7.4 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 171.2, 151.6, 144.4, 128.8, 128.0 (q, *J* = 3.8 Hz), 126.8, 126.0 (q, *J* = 33.4 Hz), 124.9 (q, *J* = 3.7 Hz), 124.6, 123.5 (q, *J* = 272.2 Hz), 123.3, 120.4, 51.2, 50.7, 45.6, 41.6, 32.1, 29.3, 27.0; Anal. calcd for $C_{19}H_{20}F_3N_2OSCl$ C, 54.74; H, 4.84; N, 6.72; found C, 54.73; H, 4.60; N, 6.58; HRMS calcd for $C_{19}H_{20}F_3N_2OSClNa$ $[M + Na]^+$ 439.0829 found 439.085.

*4.1.17 1-(4-(2-Bromo-4-(trifluoromethyl)phenyl)piperazin-1-yl)-4-(thiophen-2-yl)butan-1-one (**3j**).*[35]

Prepared from 1-(2-bromo-4-(trifluoromethyl)phenyl)piperazine **2j** (95 mg, 0.30 mmol) and 4-(2-thienyl)butyric acid (41 µL, 0.28 mmol) using HOBt (41 mg, 0.30 mmol), TBTU (98 mg, 0.30 mmol), anhydrous NEt_3 (62 µL, 0.44 mmol) and anhydrous DMF (2 mL) and following the general procedure described in method 1 section 4.1.6. The reaction mixture was stirred at room temperature for 21 h under N_2 atmosphere. DMF was removed under reduced pressure and the residue was purified using column chromatography (1:1, EtOAc:Pet. ether (40-60 ^{o}C)) to give a clear oil, 60 mg (50%). R_f: 0.8 (1:1 EtOAc:Pet. ether (40-60 ^{o}C)); IR (neat): υ (cm^{-1}) 2914, 1645, 1605, 1432, 1324, 1122, 680; ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.49 (dd, *J* = 8.3, 0.6 Hz, 1H), 7.28 – 7.24 (m, 1H), 7.22 – 7.20 (m, 1H), 7.14 (dd, *J* = 5.1, 1.2 Hz, 1H), 6.93 (dd, *J* = 5.1, 3.4 Hz, 1H), 6.84 – 6.80 (m, 1H), 3.86 – 3.79 (m, 2H), 3.63 – 3.58 (m, 2H), 3.07 – 3.02 (m, 4H), 2.94 (td, *J* = 7.3, 0.5 Hz, 2H), 2.45 – 2.38 (m, 2H), 2.11 – 2.03 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 171.3, 149.2, 144.4, 132.6 (q, *J* = 1.26 Hz), 131.2, 130.2 (q, *J* = 32.8 Hz), 126.8, 124.6, 123.7 (q, *J* = 272.9 Hz), 123.2, 120.8 (q, *J* = 3.77 Hz), 117.4 (q, *J* = 3.7 Hz), 51.3, 51.0, 45.6, 41.7, 32.1, 29.3, 27.0; HRMS calcd for $C_{19}H_{20}BrF_3N_2OSK$ $[M + K]^+$ 499.0063 found 499.0079.

*4.1.18 4-(Thiophen-3-yl)-1-(4-(5-(trifluoromethyl)pyridin-2-yl)piperazin-1-yl)butan-1-one (**5a**).*[35]

Prepared from 1-(5-(trifluoromethyl)pyridin-2-yl)piperazine (99 mg, 0.43 mmol) and 4-(2-thienyl)butyric acid (50 µL, 0.34 mmol) using HOBt (58 mg, 0.43 mmol), TBTU (140 mg, 0.43 mmol), NEt_3 (100 µL, 0.69 mmol) and DMF (3 mL) and following the general procedure described in method 2 section 4.1.7. The reaction mixture was stirred at room temperature for 20 h. DMF was removed under reduced pressure and the residue was purified using column chromatography (3:2 EtOAc:*n*-hexane) to give a white solid, 119 mg (91%). Mp: 107-109 °C; R_f: 0.5 (3:2, EtOAc:*n*-hexane); IR (ATR): υ (cm^{-1}) 2912, 1645, 1609, 1415, 1318, 1106; ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 8.41 (s, 1H), 7.66 (dd, *J* = 9.0 Hz, *J* = 2.2 Hz, 1H), 7.14 – 7.10 (m, 1H), 6.94 – 6.91 (m, 1H), 6.82 – 6.80 (m, 1H), 6.64 (d, *J* = 9.0 Hz, 1H), 3.79 – 3.68 (m, 4H), 3.66 – 3.56 (m, 2H), 3.56 – 3.49 (m, 2H), 2.93 (t, *J* = 7.4 Hz, 2H),

2.41 (t, *J* = 7.4, 2H), 2.06 (p, *J* = 7.4 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 171.3, 160.1, 145.8 (q, *J* = 4.3 Hz), 144.3, 134.7 (q, *J* = 3.2 Hz), 126.8, 124.6, 124.5 (q, *J* = 270.9 Hz), 123.2, 115.9 (q, *J* = 33.1 Hz), 105.7, 45.0, 44.7, 44.4, 41.0, 32.0, 29.3, 26.9; Anal. calcd for $C_{18}H_{20}F_3N_3OS$ C, 56.38; H, 5.26; N, 10.96; found C, 56.64; H,4.95; N, 10.89; HRMS calcd for $C_{18}H_{21}F_3N_3OS$ $[M + H]^+$ 384.1352 found 384.1355.

*4.1.19 1-(4-(6-Chloro-5-(trifluoromethyl)pyridin-2-yl)piperazin-1-yl)-4-(thiophen-2-yl)butan-1-one (**5b**).*[35]

Prepared from 1-(6-chloro-5-(trifluoromethyl)pyridin-2-yl)piperazine (218 mg, 0.816 mmol) and 4-(2-thienyl)butyric acid (100 µL, 0.68 mmol) using HOBt (110 mg, 0.82 mmol), TBTU (262 mg, 0.82 mmol), anhydrous NEt_3 (152 µL, 1.08 mmol) and anhydrous DMF (5 mL) and following the general procedure described in method 1 section 4.1.6. The reaction mixture was stirred at room temperature for 20 h under a N_2 atmosphere. DMF was removed under reduced pressure and the residue was purified using column chromatography (3:2, EtOAc:Pet. ether (40-60 ^oC)) to give a beige solid, 192 mg (68%). Mp: 62-64 °C; R_f: 0.48 (3:2, EtOAc:Pet. ether (40-60 ^oC)); IR (KBr): υ (cm^{-1}) 2904, 1678, 1641, 1416, 1321, 1120, 698; ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.70 (d, *J* = 8.8 Hz, 1H), 7.13 (dd, *J* = 5.1, 1.2 Hz, 1H), 6.92 (dd, *J* = 5.1, 3.4 Hz, 1H), 6.84 – 6.77 (m, 1H), 6.48 (d, *J* = 8.7 Hz, 1H), 3.79 – 3.66 (m, 4H), 3.64 – 3.45 (m, 4H), 2.93 (t, *J* = 7.5 Hz, 2H), 2.45 – 2.35 (m, 2H), 2.10 – 2.02 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 171.3, 159.0, 147.7, 144.3, 137.8 (q, *J* = 4.5 Hz), 126.9, 124.6, 123.3, 123.2 (q, *J* = 271.0 Hz), 113.0 (q, *J* = 33.6 Hz), 103.3, 44.8, 44.6, 44.2, 40.8, 32.0, 29.2, 26.9; HRMS calcd for $C_{18}H_{20}ClF_3N_3OSCl$ $[M + H]^+$, 418.0962 found 418.0953.

*4.1.20 1-(4-(3-Chloro-5-(trifluoromethyl)pyridin-2-yl)piperazin-1-yl)-4-(thiophen-2-yl)butan-1-one (**5c**).*[35]

Prepared from 1-(3-chloro-5-(trifluoromethyl)pyridin-2-yl)piperazine (114 mg, 0.43 mol) and 4-(2-thienyl)butyric acid (50 µL, 0.34 mmol) using HOBt (58 mg, 0.43 mmol), TBTU (140 mg, 0.43 mmol), NEt_3 (100 µL, 0.69 mmol) and DMF (3 mL) and following the general procedure described in method 2 section 4.1.7. The reaction mixture was stirred at room temperature for 19 h. DMF was removed under reduced pressure and the residue was purified using column chromatography (3:2, EtOAc:*n*-hexane) to give a solid, 123 mg (87%). Mp: 52-54 °C; Rf: 0.78 (3:2, EtOAc:n-hexane); IR (ATR): υ (cm^{-1}) 3036, 2852, 1646, 1600, 1438, 1317, 1117, 847; ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 8.40 (s, 1H), 7.79 (s, 1H), 7.14 – 7.10 (m, 1H), 6.95 – 6.89 (m, 1H), 6.83 – 6.78 (m, 1H), 3.81 – 3.74 (m, 2H), 3.60 – 3.53 (m, 2H), 3.51 – 3.44 (m, 4H), 2.93 (t, *J* = 7.2 Hz, 2H), 2.41 (t, *J* = 7.4 Hz, 2H), 2.06 (p, *J* = 7.3 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 171.2, 159.6, 144.4, 143.0 (q, *J* = 4.2 Hz), 136.1 (q, *J* = 3.3 Hz), 126.8, 124.5, 123.2 (q, *J* = 272.3 Hz), 123.2, 121.1, 120.6 (q, *J* = 33.5 Hz), 48.6, 45.2, 41.3, 32.0, 29.3, 27.0; Anal. calcd for $C_{18}H_{19}F_3N_3OSCl$ C, 51.73; H, 4.58; N, 10.06; found C, 52.17; H, 4.66; N, 9.89; HRMS calcd for $C_{18}H_{20}F_3N_3OSCl$ $[M + H]^+$ 418.0971 found 418.0963.

*4.1.21 1-(4-Cyclohexylpiperazin-1-yl)-4-(thiophen-2-yl)butan-1-one (**5d**).*

Prepared from 1-cyclohexylpiperazine (150 mg, 0.89 mmol) and 4-(2-thienyl)butyric acid (118 µL, 0.81 mmol) using HOBt (120 mg, 0.89 mmol), TBTU (285 mg, 0.89 mmol), NEt_3 (180 µL, 1.29 mmol) and DCM (6 mL) and following the general procedure described in methoid 2 section 4.1.7. The reaction mixture was stirred at room temperature for 17.5 h. DCM was removed under reduced pressure and the residue was purified using column chromatography (9:1, DCM:MeOH) to give a red solid, 213 mg (82%). Mp: 33-35 °C; Rf: 0.56 (9:1, DCM:MeOH); IR (ATR): υ (cm^{-1}) 2928, 2851, 1632, 1445; ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.11 (dd, *J* = 5.1, 1.0 Hz, 1H), 6.91 (dd, *J* = 5.1, 3.4 Hz, 1H), 6.79 (dd, *J* = 3.4, 1.0 Hz, 1H), 3.70 – 3.55 (m, 2H), 3.45 – 3.31 (m, 2H), 2.90 (t, *J* = 7.3 Hz, 2H), 2.67 – 2.40 (m, 4H), 2.40 – 2.32 (m, 2H), 2.32 – 2.25 (m, 1H), 2.07 – 1.96 (m, 2H), 1.90 – 1.73 (m, 4H), 1.69 – 1.56 (m, 1H), 1.31 – 1.14 (m, 4H), 1.14 – 1.04 (m, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 170.8, 144.5, 126.8, 124.6, 123.1, 63.6, 49.3, 48.7, 45.9, 41.9, 32.0, 29.3, 28.8, 27.1, 26.2, 25.8; HRMS calcd for $C_{18}H_{29}N_2OS$ $[M + H]^+$ 321.1995 found 321.2005.

*4.1.22 1-(4-Methylpiperazin-1-yl)-4-(thiophen-2-yl)butan-1-one (**5e**).*

Prepared from 1-methylpiperazine (71 µL, 0.65 mmol) and 4-(2-thienyl)butyric acid (85 µL, 0.59 mmol) using HOBt (87 mg, 0.65 mmol), TBTU (207 mg, 0.65 mmol), NEt_3 (90 µL, 0.94 mmol), and DCM (4 mL), and following the general procedure described in method 2 section 4.1.7. The reaction mixture was stirred at room temperature for 18 h. DCM was removed under reduced pressure and the residue was purified using column chromatography (3:2, EtOAc:*n*-hexane) to give a yellow oil, 104 mg (70 %). R_f: 0.56 (100% DCM); IR (ATR): υ (cm^{-1}) 2936, 2792, 1635, 1435, 1289; ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.11 (dd, *J* = 5.1, 1.2 Hz, 1H), 6.91 (dd, *J* = 5.1, 3.4 Hz, 1H), 6.80 (ddd, *J* = 3.4, 2.1, 1.2 Hz, 1H), 3.65 – 3.61 (m, 2H), 3.43 – 3.39 (m, 2H), 2.90 (t, *J* = 7.3 Hz, 2H), 2.38 – 2.33 (m, 6H), 2.29 (s, 3H), 2.02 (p, *J* = 7.4 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 170.9, 144.5, 126.8, 124.5, 123.1, 55.2, 54.7, 46.0, 45.4, 41.5, 32.0, 29.3, 27.0; HRMS calcd for $C_{13}H_{21}N_2OS$ $[M + H]^+$ 253.1369 found 253.1367.

*4.1.23 4-(Thiophen-2-yl)-1-(4-(4-(trifluoromethyl)phenyl)piperidin-1-yl)butan-1-one (**7a**).*[35]

Prepared from 4-(4-(trifluoromethyl)phenyl)piperidine.HCl (100 mg, 0.38 mmol) and 4-(2-thienyl)butyric acid (50 µL, 0.34 mmol) using HOBt (51 mg, 0.38 mmol), TBTU (122 mg, 0.38 mmol), NEt_3 (55 µL, 0.54 mmol) and DMF (2 mL) and following the general procedure described in method 2 section 4.1.7. The reaction mixture was stirred for 22 h at room temperature. DMF was removed under reduced pressure and the residue was purified using column chromatography (3:2, EtOAc:n-hexane) to give an off-white solid, 87 mg (67%). Mp: 48-49 °C; R_f: 0.7 (3:2, EtOAc:*n*-hexane); IR (ATR): υ (cm^{-1}) 2850, 1644, 1323, 1112; ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.56 (d, *J* = 8.0 Hz, 2H), 7.30 (d, *J* = 8.0 Hz, 2H), 7.12 (d, *J* = 5.1 Hz, 1H), 6.97 – 6.87 (m, 1H), 6.83 – 6.79 (m, 1H), 4.82 (d, *J* = 13.2 Hz, 1H), 3.92 (d, *J* = 13.5 Hz, 1H), 3.10 (t, *J* = 12.9 Hz, 1H), 2.93 (t, *J* = 7.3 Hz, 2H), 2.85 – 2.74 (m, 1H), 2.63 (t, *J* = 12.7 Hz, 1H), 2.41 (t, *J* = 7.4 Hz, 2H), 2.06 (p, *J* = 7.4 Hz, 2H), 1.93 – 1.84 (m, 2H), 1.68 – 1.52 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 170.9, 149.2, 144.5, 128.9 (q, *J* =

32.5 Hz), 127.1, 126.8, 125.6 (q, *J* = 3.8 Hz), 124.5, 124.2 (q, *J* = 272.3 Hz), 123.1, 46.0, 42.7, 42.2, 33.7, 32.6, 32.2, 29.4, 27.1; HRMS calcd for $C_{20}H_{22}F_3NOSNa$ $[M + Na]^+$ 404.1266 found 404.1279.

*4.1.24 4-(Thiophen-2-yl)-N'-(4-(trifluoromethyl)phenyl)butane hydrazide (**7b**).*

Prepared from (4-(trifluoromethyl)phenyl)hydrazine (195 mg, 1.11 mmol) and 4-(2-thienyl)butyric acid (149 µL, 1.03 mmol) using HOBt (149 mg, 1.11 mmol), TBTU (356 mg, 1.11 mmol), anhydrous NEt_3 (231 µL, 1.66 mmol) and anhydrous DMF (8 mL) and following the general procedure described in method 1 section 4.1.6. The reaction mixture was stirred at room temperature for 20 h under a N_2 atmosphere. DMF was removed under reduced pressure and the residue was purified using column chromatography (elution gradient 1:1 EtOAc:*n*-hexane to 3:2 EtOAc:*n*-hexane) to give an off-white solid , 235 mg (69%). Mp: 110-112 °C; R_f: 0.3 (1:1, EtOAc:*n*-hexane); IR (KBr): υ (cm^{-1}) 3309, 2963, 1639, 1614, 1333, 1104; ^{1}H NMR (500 MHz, DMSO-d6): δ (pm) 9.82 (d, *J* = 1.4 Hz, 1H), 8.39 (s, 1H), 7.47 (d, *J* = 8.6 Hz, 2H), 7.34 (dd, *J* = 5.1, 1.0 Hz, 1H), 6.97 (dd, *J* = 5.1, 3.4 Hz, 1H), 6.88 (m, 1H), 6.79 (d, *J* = 8.5 Hz, 2H), 2.83 (t, *J* = 7.6 Hz, 2H), 2.26 (t, *J* = 7.4 Hz, 2H), 1.95 – 1.85 (m, 2H); ^{13}C NMR (126 MHz, DMSO-d6): δ (ppm) 172.2, 153.0, 144.5, 127.5, 126.6 (q, *J* = 3.8 Hz), 126.1 (q, *J* = 294.2 Hz), 125.1, 124.1, 118.5 (q, *J* = 31.9 Hz), 111.8, 32.9, 29.1, 27.7; Anal. calcd for $C_{15}H_{15}F_3N_2OS$ C, 54.87; H, 4.60; N, 8.53; found C, 54.44; H, 4.39; N, 8.09; HRMS calcd for $C_{15}H_{16}F_3N_2OS$ $[M + H]^+$ 330.0975 found 330.096.

*4.1.25 4-(Thiophen-2-yl)-N-(4-(trifluoromethyl)phenyl)butanamide (**7c**)*

Prepared from 4-(trifluoromethyl)aniline (69 mg, 0.43 mmol) and 4-(thiophen-2-yl)butanoic acid (50 µL, 0.34 mmol) using HOBt (58 mg, 0.43 mmol), TBTU (140 mg, 0.43 mmol), NEt_3 (100 µL, 0.51 mmol) and DCM (6 mL) and following the general procedure described in method 2 section 4.1.7. The reaction mixture was stirred at room temperature for 20 h. DCM was removed under reduced pressure and the residue was purified using column chromatography (3:2, EtOAc:n-hexane) to give a white solid, 96 mg (90%). R_f: 0.6 (3:2, EtOAc:*n*-hexane); IR (ATR): υ (cm^{-1}) 3304, 2926, 1670, 1514, 1319, 1125; ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.62 (d, *J* = 8.6 Hz, 2H), 7.56 (d, *J* = 8.6 Hz, 2H), 7.29 (bs, 1H), 7.14 (ddd, *J* = 5.1, 1.2, 0.5 Hz, 1H), 6.93 (dd, *J* = 5.1, 3.4 Hz, 1H), 6.81 (dd, *J* = 2.2, 1.1 Hz, 1H), 2.94 (t, *J* = 7.2 Hz, 2H), 2.42 (t, *J* = 7.4 Hz, 2H), 2.12 (p, *J* = 7.2 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 171.0, 143.8, 140.8, 126.9, 126.3 (q, *J* = 3.8 Hz), 126.1 (q, *J* = 33.0 Hz), 124.8, 124.1 (q, *J* = 272.1 Hz), 123.4, 119.3, 36.4, 29.0, 27.0; HRMS calcd for $C_{15}H_{14}NOSNa$ $[M + H]^+$ 336.1393 found 336.1393.

*4.1.26 3-(Thiophen-2-ylthio)propanoic acid (**8f**).*

Thienyl-2-thiol (48 µl, 0.52 mmol), 3-bromopropanoic acid (80 mg, 0.52 mmol) and $NaHCO_3$ (130 mg, 1.56 mmol) were heated at reflux in EtOH (2 mL). After 5 h the reaction mixture was allowed to cool to room temperature and the EtOH was removed under reduced pressure. The residue was dissolved in H_2O (2 mL) and washed with Et_2O (4 x 10 mL). The aqueous layer was acidified to pH = 6 with 0.1 M aqueous HCl and extracted with Et_2O (4 x 10 mL). The combined organic layers were concentrated under reduced pressure and the

residue was purified using column chromatography (1:9, MeOH:DCM) to give a clear oil, 83 mg (85%). R_f: 0.3 (1:9, MeOH:DCM); ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 10.86 (br s, 1H), 7.37 (dd, *J* = 5.1, 1.2 Hz, 1H), 7.16 (dd, *J* = 2.4, 1.2 Hz, 1H), 6.97 – 6.99 (m, 1H), 2.97 (t, *J* = 7.2 Hz, 2H), 2.64 (t, *J* = 7.2 Hz, 2H); ^{1}H NMR data matches literature data [31]; HRMS calcd for $C_7H_9O_2S_2$ $[M + H]^+$: 189.0038 found 189.0040.

*4.1.27 Thiophen-2-yl (4-(4-(trifluoromethyl)phenyl)piperazin-1-yl) methanone (**9a**).*

Prepared from 1-(4-(trifluoromethyl)phenyl)piperazine **2a** (76 mg, 0.33 mmol) and thiophene-2-carboxylic acid (38 mg, 0.30 mmol) using HOBt (45 mg, 0.33 mol), TBTU (107 mg, 0.33 mmol), NEt_3 (75 µL, 0.48 mmol) and DMF (4 mL) and following the general procedure described in method 2 section 4.1.7. The reaction mixture was stirred for 21 h at room temperature. DMF was removed under reduced pressure and the residue was purified using column chromatography (1:1, EtOAc:Pet. ether (40-60 ^oC)) to give a solid, 70 mg (69%). Mp: 126-130 °C; R_f: 0.6 (1:1, EtOAc:Pet.ether (40-60 ^oC)); IR (ATR): υ (cm^{-1}) 2838, 1606, 1334, 1096; ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.55 – 7.45 (m, 3H), 7.35 (d, *J* = 3.2 Hz, 1H), 7.13 – 7.02 (m, 1H), 6.93 (d, *J* = 8.6 Hz, 2H), 3.98 – 3.86 (m, 4H), 3.42 – 3.27 (m, 4H); ^{13}C NMR (126 MHz, $CDCl_3$) δ (ppm) 163.7, 152.8, 136.6, 129.1, 129.0, 126.8, 126.5 (q, *J* = 3.8 Hz), 124.5 (q, *J* = 271.3 Hz), 121.3 (q, *J* = 32.8 Hz), 114.9, 48.2; Anal. calcd for $C_{16}H_{15}F_3N_2OS$ C, 56.46; H, 4.44; N, 8.23; found C, 56.72; H, 4.62; N, 8.42; HRMS calcd for $C_{16}H_{15}F_3N_2OSNa$ $[M + Na]^+$ 363.0749 found 363.0762.

*4.1.28 2-(Thiophen-2-yl)-1-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)ethanone (**9b**).*[35]

Prepared from 1-(4-(trifluoromethyl)phenyl)piperazine **2a** (100 mg, 0.43 mmol) and 2-(thiophen-2-yl)acetic acid (48 mg, 0.34 mmol) using HOBt (58 mg, 0.43 mmol), TBTU (138 mg, 0.43 mmol), NEt_3 (87 µL, 0.63 mmol), DMF (4 mL) and following the general procedure described in method 2 section 4.1.7. The reaction mixture was stirred at room temperature for 21 h. Purified using column chromatography (3:2, EtOAc:*n*-hexane) to give an orange solid, 98 mg (82%).]. R_f: 0.9 (3:2, EtOAc:*n*-hexane); IR (ATR): υ (cm^{-1}) 2826, 1614, 1409, 1336, 1229, 1101; ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.49 (d, *J* = 8.7 Hz, 2H), 7.21 (dd, *J* = 5.1 Hz, *J* = 1.2 Hz, 1H), 6.97 – 6.88 (m, 4H), 3.96 (s, 2H), 3.84 – 3.78 (m, 2H), 3.72 – 3.64 (m, 2H), 3.29 – 3.23 (m, 2H), 3.21 – 3.12 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 168.5, 152.8, 136.2, 127.0, 126.5 (q, *J* = 3.3 Hz), 126.1, 124.9, 124.6 (q, *J* = 271.3 Hz), 121.4 (q, *J* = 32.8 Hz), 115.0, 48.2, 47.9, 45.8, 41.5, 35.2; HRMS calcd for $C_{17}H_{17}F_3N_2O_2SNa$ $[M + Na]^+$ 377.0906 found 377.0915.

*4.1.29 3-(Thiophen-2-yl)-1-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)propan-1-one (**9c**).*[35]

Prepared from 1-(4-(trifluoromethyl)phenyl)piperazine **2a** (81 mg, 0.35 mmol) and 3-(thiophen-2-yl)propanoic acid (50 mg, 0.32 mmol) using HOBt (47 mg, 0.35 mmol), TBTU (113 mg, 0.35 mmol), anhydrous NEt_3 (71 µL, 0.51 mmol) and anhydrous DMF (5 mL) and following the general procedure described in method 1 section 4.1.6. The reaction mixture was stirred at room temperature for 16 h under a N_2 atmosphere. DMF was removed under reduced pressure and the residue was purified using column chromatography (3:2, EtOAc:*n*-

hexane) to give an orange solid, 52 mg (44%). Mp: 50-54 °C; R_f: 0.5 (3:2, EtOAc:*n*-hexane); IR (KBr): υ (cm^{-1}) 2826, 1632, 1615, 1441, 1338, 1229; ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.49 (d, *J* = 8.5 Hz, 2H), 7.12 (dd, *J* = 5.1 Hz, *J* = 1.2 Hz), 6.95 – 6.87 (m, 3H), 6.87 – 6.82 (m, 1H), 3.80 – 3.77 (m, 2H), 3.59 – 3.56 (m, 2H), 3.25 – 3.17 (m, 6H, 4H), 2.79 – 2.65 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 170.2, 152.8, 143.6, 126.9, 126.5 (q, *J* = 3.3 Hz), 124.8, 124.5 (q, *J* = 269.7 Hz), 123.5, 121.3 (q, *J* = 32.4 Hz), 115.0, 48.2, 48.0, 45.0, 41.2, 35.1, 25.5; HRMS calcd for $C_{18}H_{20}F_3N_2OS$ $[M + H]^+$ 369.1243 found: 369.1238.

*4.1.30 5-(Thiophen-2-yl)-1-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)pentan-1-one (**9d**).*[35]

Prepared from 5-(thiophen-2-yl)pentanoic acid (72 mg, 0.39 mmol) using HOBt (58 mg, 0.43 mmol), TBTU (138 mg, 0.43 mmol), anhydrous NEt_3 (86 µL, 0.62 mmol), and anhydrous DMF (4 mL) in an oven-dried Schlenk tube under a N_2 atmosphere. The resulting solution was stirred at room temperature for 15 mins. A second Schlenk tube was prepared containing 1-(4-(trifluoromethyl)phenyl)piperazine **2a** (100 mg, 0.43 mmol) and DMF (4 mL) under a N_2 atmosphere. The resulting solution was transferred, via a cannula, to the first Schlenk tube containing the carboxylic acid. The reaction mixture was stirred at room temperature for 18 h under a N_2 atmosphere. The DMF was removed under reduced pressure. The resulting oil was acidified (pH = 3) using a 0.1 M aqueous HCl solution and purified using column chromatography (3:2, EtOAc:n-hexane) to give a solid, 113 mg (73%). Mp: 46-48 °C; R_f: 0.66 (3:2, EtOAc:n-hexane); IR (KBr): υ (cm^{-1}) 2862, 1634, 1612, 1438, 1326, 1107; ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.50 (d, J = 8.8 Hz, 2H), 7.09 (dd, J = 5.1, 1.2 Hz, 1H), 6.94 – 6.86 (m, 3H), 6.81 – 6.74 (m, 1H), 3.82 – 3.70 (m, 2H), 3.66 – 3.53 (m, 2H), 3.27 – 3.20 (m, 4H), 2.87 (t, J = 6.7 Hz, 2H), 2.39 (t, J = 7.0 Hz, 2H), 1.81 – 1.68 (m, 4H); ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 171.4, 152.9, 145.0, 126.7, 126.5 (q, J = 3.8 Hz), 124.6 (q, J = 271.3 Hz), 124.2, 123.0, 121.3 (q, J = 32.9 Hz), 115.0, 48.4, 48.1, 45.1, 41.1, 33.0, 31.4, 29.7, 24.6; HRMS calcd for $C_{20}H_{24}F_3N_2OS$ $[M + H]^+$ 397.1556 found 397.1572.

*4.1.31 5-(Thiophen-2-yl)-1-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl) pentan-1-one (**9e**).*[35]

Prepared from 1-(4-(trifluoromethyl)phenyl)piperazine **2a** (100 mg, 0.43 mmol) and 5-(thiophen-2-yl)pentanoic acid (77 mg, 0.34 mmol) using HOBt (58 mg, 0.43 mmol), TBTU (138 mg, 0.43 mmol), anhydrous NEt_3 (87 µL, 0.63 mmol) and anhydrous DMF (4 mL) and following the general procedure described in method 1 section 4.1.6. The reaction mixture was stirred at room temperature for 23 h under a N_2 atmosphere. DMF was removed under reduced pressure and the residue was purified using column chromatography (4:1, EtOAc:*n*-hexane) to give a brown solid, 120 mg (75%). Mp: 50-52 °C; R_f: 0.5 (3:2, EtOAc:*n*-hexane); IR (KBr): υ (cm^{-1}) 2926, 1641, 1613, 1438, 1332, 1240; ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.49 (d, *J* = 9.1 Hz, 2H), 7.08 (dd, *J* = 5.1 Hz, *J* = 1.2 Hz, 1H), 6.92-6.88 (m, 3H), 6.77-6.76 (m, 1H), 3.78-3.75 (m, 2H), 3.62-3.59 (m, 2H), 3.27-3.22 (m, 4H), 2.83 (t, *J* = 7.2 Hz, 2H), 2.36 (t, *J* = 7.2 Hz, 2H), 1.76-1.64 (m, 4H), 1.48-1.44 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 171.5, 152.9, 145.3, 126.7, 126.5 (q, *J* = 3.3 Hz), 124.1, 122.8, 124.6 (q, *J* =

270.0 Hz), 121.7 (q, *J* = 32.0 Hz), 114.9, 48.3, 48.0, 45.1, 41.1, 33.0, 31.5, 29.7, 28.8, 24.9. HRMS calcd for $C_{42}H_{50}F_6N_4O_2S_2Na$ $[2M + Na]^+$ 843.3172 found 843.3152.

*4.1.32 3-(Thiophen-2-ylthio)-1-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)propan-1-one (**9f**).*[35]

Prepared from 1-(4-(trifluoromethyl)phenyl)piperazine **2a** (50 mg, 0.22 mmol) and 3-(thiophen-2-ylthio)propanoic acid **8f** (37 mg, 0.19 mmol) using HOBt (30 mg, 0.22 mmol), TBTU (70 mg, 0.22 mmol), NEt_3 (44 µL, 0.32 mmol) and DMF (2 mL) and following the general procedure described in method 2 section 4.1.7. The reaction mixture was stirred at room temperature for 18 h. DMF was removed under reduced pressure and the residue was purified using column chromatography (3:2, EtOAc:Pet. ether (40-60 ^oC)) to give a colourless oil, 45 mg (60%). Mp: 86-90 °C; R_f: 0.75 (3:2, EtOAc:Pet. ether (40-60 ^oC)); IR (ATR): υ (cm^{-1}) 2837, 1640, 1614, 1331, 1103; ^1H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.50 (d, *J* = 8.7 Hz, 2H), 7.36 (dd, *J* = 5.4, 1.2 Hz, 1H), 7.15 (dd, *J* = 3.6, 1.2 Hz, 1H), 6.99 (dd, *J* = 5.4, 3.6 Hz, 1H), 6.91 (d, *J* = 8.7 Hz, 2H), 3.81 – 3.74 (m, 2H), 3.61 – 3.54 (m, 2H), 3.30 – 3.23 (m, 4H), 3.12 (t, *J* = 7.3 Hz, 2H), 2.68 (t, *J* = 7.3 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 169.4, 152.9, 134.0, 134.0, 129.6, 127.7, 126.5 (q, *J* = 3.8 Hz), 124.6 (q, *J* = 271.3 Hz), 121.4 (q, *J* = 32.8 Hz), 115.0, 48.3, 48.1, 45.0, 41.3, 34.1, 33.2; HRMS calcd for $C_{18}H_{19}F_3N_2OS_2Na$ $[M + Na]^+$ 423.0783 found 423.0797.

*4.1.33 1-(Thiophen-2-yl)-4-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)butane-1,4-dione (**9g**).*[35]

Prepared from 1-(4-(trifluoromethyl)phenyl)piperazine **2a** (100 mg, 0.43 mmol) and 4-oxo-4-(thiophen-2-yl)butanoic acid (63 mg, 0.34 mmol), using HOBt (58 mg, 0.43 mmol), TBTU (140 mg, 0.43 mmol), NEt_3 (100 µL, 0.69 mmol) and DMF (3 mL) and following the general procedure described in method 2 section 4.1.7. The reaction mixture was stirred at room temperature for 17 h. DMF was removed under reduced pressure and the residue was purified using column chromatography (3:2 EtOAc:*n*-hexane) to give an off-white solid, 114 mg (84%). Mp: 172-176 °C; R_f: 0.5 (3:2, EtOAc:*n*-hexane); IR (ATR): υ (cm^{-1}) 2948, 1647, 1612, 1325, 1223; ^1H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.81 (d, *J* = 3.6 Hz, 1H), 7.63 (d, *J* = 4.9 Hz, 1H), 7.50 (d, *J* = 8.6 Hz, 2H), 7.13 (t, *J* = 4.3 Hz, 1H), 6.92 (d, *J* = 8.6 Hz, 2H), 3.83 – 3.70 (m, 4H), 3.34 – 3.32 (m, 4H), 3.27 – 3.25 (m, 2H), 2.82 (t, *J* = 6.5 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 191.9, 170.1, 152.9, 143.9, 133.6, 132.1, 128.2, 126.5 (q, *J* = 3.8 Hz), 124.6 (q, *J* = 271.3 Hz), 121.2 (q, *J* = 32.6 Hz), 115.0, 48.2, 48.0, 45.0, 41.4, 34.2, 27.0; Anal. calcd for $C_{19}H_{19}F_3N_2O_2S$ C, 57.56; H, 4.83; N, 7.07; found C, 57.34; H, 4.62; N, 6.89; HRMS calcd for $C_{19}H_{20}F_3N_2O_2S$ $[M + H]^+$ 397.1192 found 397.1179.

*4.1.34 4-(Thiophen-2-yl)butan-1-ol (**10**).*

To a suspension of $LiAlH_4$ (130 mg, 3.43 mmol) in THF (2.5 mL) was added a solution of 4-(2-thienyl)butyric acid (500 µL, 3.43 mmol) in THF (2.5 mL). The suspension was stirred for 6 h at room temperature. The reaction mixture was quenched with MeOH (2 mL) and 10% aqueous NaOH (2 mL). The solution was neutralised with 10% aqueous HCl (2 mL) and the aqueous layer extracted with EtOAc (4 x 10 mL). The combined organic layers were dried

over $MgSO_4$ and the residue purified using column chromatography (DCM) to give a colourless oil, 481 mg (90%). R_f: 0.2 (DCM); IR (ATR): υ (cm^{-1}) 3329, 2934; ^1H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.11 – 7.05 (m, 1H), 6.92 – 6.87 (m, 1H), 6.79 – 6.74 (m, 1H), 3.60 (t, *J* = 6.6 Hz, 2H), 2.83 (t, *J* = 7.6 Hz, 2H), 2.35 (bs, 1H), 1.77 – 1.68 (m, 2H), 1.64 – 1.55 (m, 2H); ^1H NMR matches literature data [35]; ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 145.3, 126.8, 124.2, 123.0, 62.4, 32.1, 29.7, 28.0; HRMS calcd for $C_8H_{12}OSNa$ $[M + Na]^+$ 179.0501 found 179.0509.

*4.1.35 4-(Thiophen-2-yl)butyl methanesulfonate (**11**).*

Methanesulfonyl chloride (71 µL, 0.91 mmol) was added to a solution of 4-(thiophen-2-yl)butan-1-ol **10** (135 mg, 0.86 mmol) and NEt_3 (142 µL, 1.01 mmol) in anhydrous DCM (3 mL) kept at 0 ºC. The reaction mixture was maintained at 0 °C for 1 hr, followed by warming to room temperature, where it was kept under vigorous stirring and a N_2 atmosphere for 3 h. The solvent was removed under reduced pressure and purified using column chromatography (3:2, EtOAc:Pet. ether (40-60 ^oC)) to give a colourless oil, 152 mg (75%). R_f: 0.7 (3:2, EtOAc:Pet.ether (40-60 ^oC)); IR (ATR): υ (cm^{-1}) 2938, 1348, 1169, 930; ^1H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.18 – 7.07 (m, 1H), 6.95 – 6.88 (m, 1H), 6.81 – 6.76 (m, 1H), 4.27 – 4.20 (m, 2H), 2.98 (s, 3H), 2.92 – 2.83 (m, 2H), 1.87 – 1.75 (m, 4H); ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 144.3, 126.8, 124.5, 123.2, 69.6, 37.4, 29.2, 28.4, 27.6; HRMS calcd for $C_9H_{14}O_3S_2Na$ $[M + Na]^+$ 257.0277 found 257.0271.

*4.1.36 1-(4-(Thiophen-2-yl)butyl)-4-(4-(trifluoromethyl)phenyl)piperazine (**12**).*

To a suspension of 4-(thiophen-2-yl)butyl methanesulfonate **11** (29 mg, 0.12 mmol) and Na_2CO_3 (26 mg, 0.24 mmol) in acetonitrile (5 mL), was added 1-(4-(trifluoromethyl)phenyl)piperazine **2a** (110 mg, 0.48 mmol). The mixture was heated at reflux for 24 h under vigorous agitation and a N_2 atmosphere. The solvent was removed under reduced pressure and the residue was purified using column chromatography (3:2, EtOAc:*n*-hexane) to give a white solid, 40 mg (91%). Mp: 78-80 °C; R_f: 0.8 (3:2, EtOAc:*n*-hexane); IR (ATR): υ (cm^{-1}) 2839, 1613, 1325, 1099; ^1H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.48 (d, *J* = 8.7 Hz, 2H), 7.12 – 7.10 (m, 1H), 6.94 – 6.90 (m, 3H), 6.81 – 6.78 (m, 1H), 3.32 – 3.24 (m, 4H), 2.87 (t, *J* = 7.5 Hz, 2H), 2.62 – 2.54 (m, 4H), 2.42 (t, *J* = 7.5 Hz, 2H), 1.74 (dt, *J* = 15.2, 7.5 Hz, 2H), 1.66 – 1.57 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 153.3, 145.3, 126.7, 126.3 (q, *J* = 3.3 Hz), 124.7 (q, *J* = 269.4 Hz), 124.1, 122.9, 120.4 (q, *J* = 32.4 Hz), 114.4, 58.3, 53.0, 48.0, 29.8, 29.7, 26.3; HRMS calcd $C_{19}H_{24}F_3N_2S$ $[M + H]^+$ 369.1067 found 369.1608.

*4.1.37 1-(4-(4-(Trifluoromethyl)phenyl)piperazin-1-yl)pentan-1-one (**14a**).*[35]

Prepared from 1-(4-(trifluoromethyl)phenyl)piperazine **2a** (246 mg, 1.07 mmol) and pentanoic acid (106 µL, 0.98 mmol) using HOBt (144 mg, 0.98 mmol), TBTU (343 mg, 0.98 mmol), anhydrous NEt_3 (216 µL, 1.56 mmol) and anhydrous DMF (6 mL) and following the general procedure described in method 1 section 4.1.6. The reaction mixture was stirred at room temperature for 23 h under a N_2 atmosphere. DMF was removed under reduced pressure and the residue was purified using column chromatography (3:2, EtOAc:*n*-hexane)

to give an off-white solid , 178 mg (57%). Mp: 46-48 °C; R_f: 0.6 (3:2, EtOAc:*n*-hexane); IR (KBr): υ (cm^{-1}) 2935, 1650, 1631, 1450, 1337, 1228; ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.49 (d, *J* = 8.7 Hz, 2H), 6.92 (d, *J* = 8.7 Hz, 2H), 3.79-3.76 (m, 2H), 3.66-3.62 (m, 2H), 3.30-3.23 (m, 4H), 2.37 (t, *J* = 7.2 Hz, 2H), 1.69-1.59 (m, 2H), 1.45-1.32 (m, 2H), 0.94 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 171.8, 152.9, 126.4 (q, *J* = 3.3 Hz), 124.6 (q, *J* = 268.8 Hz), 121.0 (q, *J* = 32.7 Hz), 114.9, 48.3, 48.0, 45.1, 41.0, 33.0, 27.4, 22.6, 13.9; HRMS calcd for $C_{16}H_{22}F_3N_2O$ $[M + H]^+$ 315.1679 found 315.1688.

*4.1.38 4-(1H-Pyrazol-4-yl)-1-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)butan-1-one (**14b**).*[35]

Prepared from 1-(4-(trifluoromethyl)phenyl)piperazine **2a** (100 mg, 0.43 mmol) and 4-(1*H*-pyrazol-4-yl)butanoic acid (52 mg, 0.34 mmol), using HOBt (58 mg, 0.43 mmol), TBTU (140 mg, 0.43 mmol), NEt_3 (100 µL, 0.69 mmol) and DCM (3 mL) and following the general procedure described in method 2 section 4.1.7. The reaction mixture was stirred at room temperature for 24 h. DCM was removed under reduced pressure and the residue was purified using column chromatography (3:2, EtOAc:*n*-hexane) to give a brown solid, 116 mg (93%). R_f: 0.1 (3:2, EtOAc:n-hexane); IR (ATR): υ (cm^{-1}) 3055, 2947, 1649, 1614, 1226; ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.50 (d, *J* = 8.5 Hz, 2H), 7.43 (s, 2H), 6.91 (d, *J* = 8.6 Hz, 2H), 5.57 (bs, 1H), 3.85 – 3.72 (m, 2H), 3.64 – 3.54 (m, 2H), 3.26 (s, 4H), 2.59 (t, *J* = 7.4 Hz, 2H), 2.44 – 2.35 (m, 2H), 2.00 – 1.90 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 171.4, 152.9, 132.9, 126.5 (q, *J* = 3.9 Hz), 124.6 (q, *J* = 271.3 Hz), 121.4 (q, *J* = 32.6 Hz), 120.3, 115.0, 48.4, 48.1, 45.1, 41.2, 32.3, 26.2, 23.6; HRMS calcd for $C_{18}H_{21}F_3N_4ONa$ $[M + Na]^+$ 389.156 found 389.1563.

*4.1.39 1-(4-(4-(Trifluoromethyl)phenyl)piperazin-1-yl)ethanone (**16a**).*[35]

Prepared from 1-(4-(trifluoromethyl)phenyl)piperazine **2a** (150 mg, 0.65 mmol) and acetic acid (33 µL, 0.59 mmol) using HOBt (87 mg, 0.65 mmol), TBTU (208 mg, 0.65 mmol), NEt_3 (131 µL, 0.94 mmol), and DMF (3 mL) and following the general procedure described in method 2 section 4.1.7. The reaction mixture was stirred at room temperature for 23 h. DMF was removed under reduced pressure and the residue was purified using column chromatography (4:1, EtOAc:*n*-hexane) to give an off-white solid, 117 mg (73%). Mp: 76-80 °C; R_f: 0.5 (4:1, EtOAc:*n*-hexane); IR (ATR): υ (cm^{-1}) 3222, 1614, 1428, 1324, 1105; ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.50 (d, *J* = 8.6 Hz, 2H), 6.92 (d, *J* = 8.7 Hz, 2H), 3.84 – 3.71 (m, 2H), 3.70 – 3.57 (m, 2H), 3.35 – 3.19 (m, 4H), 2.14 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 169.1, 152.9, 126.5 (q, *J* = 3.3 Hz), 124.6 (q, *J* = 277.2 Hz), 121.2 (q, *J* = 32.8 Hz), 115.0, 48.3, 48.0, 45.8, 41.0, 21.3; Anal. calcd for $C_{13}H_{15}F_3N_2O$ C, 57.34; H, 5.55; N, 10.29; found C, 57.16; H, 5.40; N, 9.87; HRMS calcd for $C_{13}H_{15}F_3N_2ONa$ $[M + Na]^+$ 295.1029 found 295.1037.

*4.1.40 1-(4-(4-(Trifluoromethyl)phenyl)piperazin-1-yl)octan-1-one (**16b**).*[35]

Prepared from 1-(4-(trifluoromethyl)phenyl)piperazine **2a** (100 mg, 0.43 mmol) and octanoic acid (62 µL, 0.39 mmol) using HOBt (58 mg, 0.43 mmol), TBTU (138 mg, 0.43 mmol), anhydrous NEt_3 (87 µL, 0.63 mmol), and anhydrous DMF (4 mL) and following the general

procedure described in method 1 section 4.1.6. The reaction mixture was stirred at room temperature for 22 h under a N_2 atmosphere. DMF was removed under reduced pressure and the residue was purified using column chromatography (3:2, EtOAc:*n*-hexane) to give a cloudy white oil, 88 mg (63%). R_f: 0.7 (3:2, EtOAc:*n*-hexane); IR (neat): υ (cm^{-1}) 2927, 1641, 1616, 1436, 1331, 1116; ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.49 (d, *J* = 8.7 Hz, 2H), 6.91 (d, *J* = 8.7 Hz, 2H), 3.80 – 3.72 (m, 2H), 3.67 – 3.56 (m, 2H), 3.30 – 3.20 (m, 4H), 2.39 – 2.31 (m, 2H), 1.70 – 1.59 (m, 2H), 1.38 – 1.24 (m, 8H), 0.92 – 0.82 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 171.8, 152.9, 126.4 (q, *J* = 3.3 Hz), 124.6 (q, *J* = 269.5 Hz), 121.2 (q, *J* = 32.7 Hz), 115.0, 48.4, 48.1, 45.2, 41.1, 33.3, 31.7, 29.4, 29.1, 25.3, 22.6, 14.1; HRMS calcd for $C_{19}H_{28}F_3N_2O$ $[M + H]^+$ 357.2148 found 357.2136.

*4.1.41 5-Phenyl-1-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)pentan-1-one (**16c**).*[35]

Prepared from 1-(4-(trifluoromethyl)phenyl)piperazine **2a** (150 mg, 0.65 mmol) and 5-phenylpentanoic acid (105 mg, 0.59 mmol) using HOBt (87 mg, 0.65 mmol), TBTU (208 mg, 0.65 mmol), NEt_3 (131 µL, 0.94 mmol), and DMF (5mL) and following the general procedure described in method 2 section 4.1.7. The reaction mixture was stirred at room temperature for 21 h. DMF was removed under reduced pressure and the residue was purified using column chromatography (4:1, EtOAc:*n*-hexane) to give an orange oil, 150 mg (65%). Mp: 72-76 °C; R_f: 0.7 (4:1, EtOAc:*n*-hexane); IR (ATR): υ (cm^{-1}) 2857, 1636, 1610, 1436, 1331, 1068; ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.48 (d, *J* = 8.7 Hz, 2H), 7.26 – 7.23 (m, 2H), 7.19 – 7.13 (m, 3H), 6.88 (d, *J* = 8.7 Hz, 2H), 3.81 – 3.68 (m, 2H), 3.62 – 3.49 (m, 2H), 3.24 – 3.17 (m, 4H), 2.68 – 2.61 (m, 2H), 2.40 – 2.32 (m, 2H), 1.75 – 1.65 (m, 4H); ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 171.5, 153.0, 142.2, 128.5, 128.4, 126.5 (q, *J* = 3.8 Hz), 125.8, 124.7 (q, *J* = 271.3 Hz), 121.2 (q, *J* = 32.8 Hz), 115.0, 48.3, 48.1, 45.1, 41.1, 35.7, 33.1, 31.1, 24.9; Anal. calcd for $C_{22}H_{25}F_3N_2O$ C, 67.67; H, 6.45; N, 7.18; found C, 67.61; H, 6.51; N, 7.18; HRMS calcd for $C_{22}H_{26}F_3N_2O$ $[M + H]^+$ 391.1992 found 391.1996.

*4.1.42 3-(Furan-2-yl)-1-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)propan-1-one (**16d**).*[35]

Prepared from 1-(4-(trifluoromethyl)phenyl)piperazine **2a** (100 mg, 0.43 mmol) and 3-(furan-2-yl)propanoic acid (54mg, 0.39 mmol) using HOBt (58 mg, 0.43 mmol), TBTU (138 mg, 0.43 mmol), anhydrous NEt_3 (87 µL, 0.63 mmol), and anhydrous DMF (4 mL) and following the general procedure described in method 1 section 4.1.6. The reaction mixture was stirred at room temperature for 22 h under a N_2 atmosphere. DMF was removed under reduced pressure and the residue was purified using column chromatography (3:2, EtOAc:*n*-hexane) to give an orange solid, 43 mg (31%). Mp: 77-81 °C; R_f: 0.6 (3:2, EtOAc:*n*-hexane); IR (KBr): υ (cm^{-1}) 2921, 1625, 1616, 1443, 1334, 1227; ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.50 (d, *J* = 8.6 Hz, 2H), 7.31 – 7.30 (m, 1H), 6.91 (d, *J* = 8.6 Hz, 2H), 6.29 – 6.27 (m, 1H), 6.05 – 6.04 (m, 1H), 3.80 – 3.79 (m, 2H), 3.61 – 3.58 (m, 2H), 3.26 – 3.21 (m, 4H), 3.08 – 2.97 (m, 2H), 2.76 – 2.67 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 170.4, 154.6, 152.9, 141.1, 126.5 (q, *J* = 3.7 Hz), 124.5 (q, *J* = 269.2 Hz), 121.3 (q, *J* = 32.5 Hz), 115.0, 110.4, 105.6, 48.3, 48.1, 45.0, 41.2, 31.6, 23.8; HRMS calcd for $C_{18}H_{20}F_3N_2O_2$ $[M + H]^+$ 353.1471 found 353.1461.

*4.1.43 9-Oxo-9-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)nonanoic acid (**16e**).*[35]

Prepared from methyl 9-oxo-9-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)nonanoate **16f** (131 mg, 0.31 mmol) and KOH (88 mg, 1.55 mmol) in EtOH (5 mL) heated at reflux for 3 h. The solution was allowed to cool to room temperature and EtOH was removed under reduced pressure. The residue was dissolved in H_2O (1 mL), the pH adjusted to pH = 6 with 2 M aqueous HCl and the aqueous layer extracted with DCM (20 mL, followed by 4 x 10 mL). The organic layers were washed with brine (3 x 20 mL), dried over $MgSO_4$ and evaporated under reduced pressure to give a white solid, 99 mg (80%). Mp: 90-92 °C; R_f: 0.5 (1:19, MeOH:DCM); IR (KBr): υ (cm^{-1}) 2935, 1706, 1617, 1333, 1204; ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.50 (d, *J* = 8.6 Hz, 2H), 6.93 (d, *J* = 8.7 Hz, 2H), 3.82 – 3.75 (m, 2H), 3.67 – 3.61 (m, 2H), 3.32 – 3.23 (m, 4H), 2.40 – 2.29 (m, 4H), 1.70 – 1.58 (m, 4H), 1.39 – 1.31 (m, 6H); ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 178.3, 172.0, 153.1, 126.7 (q, *J* = 3.8 Hz), 123.5 (q, *J* = 269.1 Hz), 120.3 (q, *J* = 32.7 Hz), 115.2, 48.6, 48.3, 45.4, 41.3, 34.0, 33.4, 29.3, 29.1, 29.0, 25.3, 24.8; Anal. calcd for $C_{20}H_{27}F_3N_2O_3$ C, 59.99; H, 6.80; N, 7.00; found C, 60.40; H, 6.83; N, 6.85; HRMS calcd for $C_{20}H_{28}F_3N_2O_3$ $[M + H]^+$ 401.2407 found 401.2047.

*4.1.44 Methyl 9-oxo-9-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)nonanoate (**16f**).*[35]

Methyl hydrogen azelate (479 mg, 2.30 mmol), BOP (1140 mg, 2.60 mmol), anhydrous NEt_3 (512 µL, 3.60 mmol), and anhydrous DCM (20 mL) were placed in an oven-dried three neck flask under a N_2 atmosphere. The resulting solution was stirred at room temperature for 15 mins. 1-(4-(trifluoromethyl)phenyl)piperazine **2a** (600 mg, 2.60 mmol) was added and the reaction mixture stirred under a N_2 atmosphere and monitored by TLC. After 16 h, DCM was removed under reduced pressure and the resulting oil was acidified to pH = 3 using a 0.1 M aqueous HCl. The aqueous mixture was extracted with DCM (20 mL, followed by 4 x 10 mL) and the organic layer washed with a saturated aqueous solution of $NaHCO_3$ (3 x 20 mL) and brine (3 x 20 mL). The organic layer was dried over $MgSO_4$ and the solvent removed *in vacuo* and the residue was purified using column chromatography (3:2, EtOAc:Pet. ether (40-60 ^{o}C)) to give an off-white solid, 952 mg (98%). Mp: 81-85 °C; R_f: 0.65 (3:2 EtOAc:Pet. ether (40-60 ^{o}C)); IR (KBr): υ (cm^{-1}) 2954, 1739, 1647, 1613, 1447, 1334, 1160; ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.49 (d, J = 8.6 Hz, 2H), 6.92 (d, J = 8.7 Hz, 2H), 3.85 – 3.71 (m, 2H), 3.72 – 3.52 (m, 5H), 3.39 – 3.14 (m, 4H), 2.32 (dt, J = 29.1, 7.5 Hz, 4H), 1.69 – 1.58 (m, 4H), 1.34 (bs, 6H); ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 174.3, 171.7, 149.2, 126.5 (q, J = 3.3 Hz), 124.5 (q, J = 269.7 Hz), 121.2 (q, J = 32.8 Hz), 115.0, 51.5, 48.5, 48.2, 45.2, 41.1, 34.1, 33.2, 29.3, 29.1, 29.0, 25.2, 24.9; HRMS calcd for $C_{21}H_{30}F_3N_2O_3$ $[M + H]^+$ 415.2203 found 415.2217.

*4.1.45 N-(2-Methoxyethyl)-9-oxo-9-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)nonanamide (**16g**).*[35]

Prepared from 9-oxo-9-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)nonanoic acid **16e** (137 mg, 0.34 mmol) and 2-methoxyethanamine (29 µL, 0.43 mmol) using HOBt (58 mg, 0.43 mmol), TBTU (140 mg, 0.43 mmol), anhydrous NEt_3 (100 µL, 0.69 mmol) and anhydrous DMF (2 mL) and following the general procedure described in method 1 section 4.1.6. The

reaction mixture was stirred at room temperature under a N_2 atmosphere for 20 h. DMF was removed under reduced pressure and the residue was purified using column chromatography (elution gradient 1:1 EtOAc:*n*-hexane to EtOAc:*n*-hexane 4:1) to give a white solid, 52 mg (34%). Mp: 88-92 °C; R_f: 0.2 (4:1, EtOAc:*n*-hexane); IR (KBr): υ (cm^{-1}) 2932, 1676, 1636, 1618, 1442, 1344, 1208; ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.50 (d, *J* = 8.5 Hz, 2H), 6.92 (d, *J* = 8.6 Hz, 2H), 5.81 (bs, 1H), 3.81 – 3.73 (m, 2H), 3.66 – 3.58 (m, 2H), 3.48 – 3.40 (m, 4H), 3.35 (s, 3H), 3.31 – 3.23 (m, 4H), 2.39 – 2.32 (m, 2H), 2.19 – 2.14 (m, 2H), 1.68 – 1.59 (m, 4H), 1.39 – 1.30 (m, 6H); ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 173.1, 171.7, 152.9, 126.5 (q, *J* = 3.8 Hz), 124.6 (q, *J* = 271.3 Hz), 121.3 (q, *J* = 32.8 Hz), 115.0, 71.3, 58.7, 48.4, 48.1, 45.2, 41.1, 39.1, 36.7, 33.2, 29.2, 29.1, 29.0, 25.6, 25.2; HRMS calcd for $C_{23}H_{35}F_3N_3O_3$ $[M + H]^+$ 458.2625 found 458.2631.

*4.1.46 1-Methyl-4-(4-(trifluoromethyl)phenyl)piperazine (**17**).*

Prepared from 1-(4-(trifluoromethyl)phenyl)piperazine **2a** (70 mg, 0.30 mmol) dissolved in absolute EtOH (3 mL) before formic acid (200 µL, 5.23 mmol) and formaldehyde (147 µL, 1.54 mmol) were consecutively added. The solution was heated at reflux for 3 h after which the EtOH was removed under reduced pressure and the residue dissolved in minimal H_2O (2 mL). The aqueous solution was neutralised to pH ~ 13 using saturated aqueous $NaHCO_3$ (4 mL) and extracted with DCM (4 x 10 mL). The organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified using column chromatography (1:1, EtOAc: Pet. ether (40-60 ^{o}C)) to give a white solid, 51 mg (70%). R_f: 0.1 (1:1, EtOAc:Pet. ether (40-60 ^{o}C)); IR (ATR): υ (cm^{-1}) 2851, 1614, 1104; ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.47 (d, *J* = 8.7 Hz, 2H), 6.92 (d, *J* = 8.7 Hz, 2H), 3.33 – 3.26 (m, 4H), 2.59 – 2.52 (m, 4H), 2.35 (s, 3H); ^{1}H NMR data matches literature data [36]; ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 153.3, 126.4 (q, *J* = 3.8 Hz), 124.8 (q, *J* = 271.1 Hz), 120.5 (q, *J* = 32.7 Hz), 114.5, 54.8, 48.0, 46.1; ^{13}C NMR data matches literature data [36]; HRMS calcd for $C_{12}H_{16}F_3N_2$ $[M + H]^+$ 245.1260 found 245.1257.

*4.1.47 1-(Thiophen-2-yl)-4-(4-(4-(trifluoromethyl)phenyl)piperidin-1-yl)butane-1,4-dione (**18**).*[35]

Prepared from 4-(4-trifluoromethyl)phenyl)piperidine (99 mg, 0.43 mmol) and 4-oxo-4- (2-thienyl)butanoic acid (63 mg, 0.34 mmol) using HOBt (58 mg, 0.43 mmol), TBTU (140 mg, 0.43 mmol), NEt3 (100 µL, 0.51 mmol), and DCM (6 mL) and following the general procedure described in method 2 section 4.1.7. The reaction mixture was stirred at room temperature for 19 h. DCM was removed under reduced pressure and the residue was purified using column chromatography (4:1, EtOAc:n-hexane) to give a white solid, 117 mg (87%). Rf: 0.3 (4:1, EtOAc:*n*-hexane); IR (ATR): υ (cm^{-1}) 2935, 1657, 1633, 1330, 1110; ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.82 (d, *J* = 3.2 Hz, 1H), 7.63 (d, *J* = 4.9 Hz, 1H), 7.57 (d, *J* = 8.1 Hz, 2H), 7.32 (d, *J* = 8.1 Hz, 2H), 7.17 – 7.08 (m, 1H), 4.79 (d, *J* = 11.6 Hz, 1H), 4.13 (d, *J* = 14.3 Hz, 1H), 3.37 – 3.28 (m, 2H), 3.23 – 3.14 (m, 1H), 2.90 – 2.77 (m, 3H), 2.71 – 2.60 (m, 1H), 1.91 (dd, *J* = 31.5, 13.1 Hz, 2H), 1.75 – 1.57 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 192.2, 169.9, 149.2, 144.1, 133.6, 132.2, 128.9 (q, *J* = 32.4 Hz), 128.2, 127.2, 125.6

(q, *J* = 3.8 Hz), 124.3 (q, *J* = 272.4 Hz), 46.0, 42.8, 42.5, 34.4, 33.6, 32.7, 27.3; HRMS calcd for $C_{20}H_{21}F_3NO_2S$ $[M + H]^+$ 396.124 found 396.1251.

*4.1.48 1-(Thiophen-2-yl)-4-(4-(5-(trifluoromethyl)pyridin-2-yl)piperazin-1-yl)butane-1,4-dione (**19**).*[35]

Prepared from 1-(5-(trifluoromethyl)pyridin-2-yl)piperazine (99 mg, 0.43 mmol) and 4-oxo-4-(thiophen-2-yl)butanoic acid (63 mg, 0.34 mmol), using HOBt (58 mg, 0.43 mmol), TBTU (140 mg, 0.43 mmol), anhydrous NEt_3 (100 µL, 0.69 mmol), and anhydrous DMF (3 mL) and following the general procedure described in method 1 section 4.1.6. The reaction mixture was stirred at room temperature for 17 h under a N_2 atmosphere. DMF was removed under reduced pressure and the residue was purified using column chromatography (3:2 EtOAc:*n*-hexane) to give an off-white solid, 85 mg, (63%). Mp.: 204-206 °C; R_f: 0.36 (3:2, EtOAc:Pet. ether (40-60 ^oC)); IR (KBr): υ (cm^{-1}) 1655, 1638, 1329, 1102; ^1H NMR (500 MHz, $CDCl_3$): δ (ppm) 8.42 (s, 1H), 7.81 (dd, *J* = 3.8, 1.1 Hz, 1H), 7.67 (dd, *J* = 9.0, 2.4 Hz, 1H), 7.63 (dd, *J* = 4.9, 1.1 Hz, 1H), 7.14 (dd, *J* = 4.9, 3.8 Hz, 1H), 6.66 (d, *J* = 9.0 Hz, 1H), 3.83 – 3.69 (m, 6H), 3.67 – 3.60 (m, 2H), 3.34 (t, *J* = 6.5 Hz, 2H), 2.82 (t, *J* = 6.5 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 191.9, 170.4, 160.0, 145.5 (q, *J* = 4.3 Hz), 143.9, 134.8 (q, *J* = 3.2 Hz), 133.6, 132.1, 128.2, 124.4 (q, *J* = 270.9 Hz), 115.8 (q, *J* = 33.2 Hz), 105.9, 44.9, 44.7, 44.4, 41.2, 34.2, 27.1; Anal. calcd for $C_{18}H_{18}F_3N_3O_2S$ C, 54.40; H, 4.57; N, 10.58; found C, 54.39; H, 4.27; N, 10.59. HRMS calcd for $C_{36}H_{37}F_6N_6O_4S_2$ $[2M + H]^+$ 795.2216 found 795.2223.

*4.1.49 1-(4-(3-Chloro-5-(trifluoromethyl)pyridin-2-yl)piperazin-1-yl)-4-(thiophen-2-yl)butane-1,4-dione (**20**).*[35]

Prepared from 1-(3-chloro-5-(trifluoromethyl)pyridin-2-yl)piperazine (114 mg, 0.43 mmol) and 4-oxo-4-(thiophen-2-yl)butanoic acid (63 mg, 0.34 mmol), using HOBt (58 mg, 0.43 mmol), TBTU (140 mg, 0.43 mmol), anhydrous NEt_3 (100 µL, 0.69 mmol), and anhydrous DMF (3 mL) and following the general procedure described in method 1 section 4.1.6. The reaction mixture was stirred at room temperature for 18 h under a N_2 atmosphere. DMF was removed under reduced pressure and the residue was purified using column chromatography (3:2 EtOAc:*n*-hexane) to give an off-white solid, 102 mg (70%). Mp: 102-104 °C; R_f: 0.5 (3:2, EtOAc:*n*-hexane); IR (KBr): υ (cm^{-1}) 2915, 1647, 1604, 1416, 1319, 1231, 844; ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 8.41 – 8.40 (m, 1H), 7.82 – 7.79 (m, 2H), 7.64 (dd, *J* = 4.0 Hz, *J* = 1.0 Hz, 1H), 7.15 – 7.12 (m, 1H), 3.80 – 3.72 (m, 4H), 3.57 – 3.54 (m, 2H), 3.50 – 3.47 (m, 2H), 3.33 (t, *J* = 9.0 Hz, 2H), 2.84 (t, *J* = 9.0 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 191.9, 170.2, 159.5, 143.9, 143.1 (q, *J* = 3.7 Hz), 136.1 (q , *J* = 3.3 Hz), 133.5, 132.1, 128.6 (q, *J* = 270.1 Hz), 128.1, 121.1 (q, *J* = 33.2 Hz), 121.0, 48.5, 45.1, 41.5, 34.2, 27.1; Anal. calcd for $C_{18}H_{17}F_3N_3O_2SCl$ C, 50.06; H, 3.97; N, 9.73; found C, 50.14; H, 3.77; N, 9.47; HRMS calcd for $C_{18}H_{18}F_3N_3O_2SCl$ $[M + H]^+$ 432.0755 found 432.0765.

*4.1.50 1-(4-(3-Chloro-5-(trifluoromethyl)pyridin-2-yl)piperazin-1-yl)-3-(thiophen-2-ylthio)propan-1-one (**21**) (RTB70) [10, 35].*

Literature procedure [10]. Prepared from 1-(3-chloro-5-(trifluoromethyl)pyridin-2-yl)piperazine (698 mg, 2.63 mmol) and 3-(thiophen-2-ylthio)propanoic acid **8f** (450 mg, 2.39 mmol) using HOBt (698 mg, 2.63 mmol), TBTU (767 mg, 2.63 mmol), anhydrous NEt_3 (532 µL, 3.82 mmol) and anhydrous DMF (10 mL) and following the general procedure described in method 1 section 4.1.6. The reaction mixture was stirred at room temperature for 21 h under a N_2 atmosphere. DMF was removed under reduced pressure and the residue was purified using column chromatography (3:2 EtOAc:*n*-hexane). The obtained product was dissolved in a minimal amount of DCM and Pet. ether (40-60 ^oC) was added until precipitation began. The solution and the precipitate were stored at -20 °C for 21 h, and the resultant crystals collected by vacuum filtration and washed with cold Pet. ether (40-60 ^oC) (~ 20 mL) to give white needles, 2.1 g (71%). ^1H NMR: (300 MHz, $CDCl_3$) δ 8.41 – 8.40 (m, 1H, H2), 7.79 – 7.78 (m, 1H, H4), 7.35 (dd, *J* = 5.3 Hz, *J* = 1.2 Hz, 1H, H10), 7.15 (m, 1H, H8), 7.00 – 6.97 (m, 1H, H9), 3.78 – 3.75 (m, 2H*), 3.58 – 3.55 (m, 2H*), 3.50 – 3.46 (m, 4H*), 3.11 (t, *J* = 7.1 Hz, 2H, H6), 2.68 (t, *J* = 7.1 Hz, 2H, H5) [10]; ^{13}C NMR: (75 MHz, $CDCl_3$) δ 170.1 (C=O), 160.1 (C3), 143.6 (q, *J* = 4.1 Hz, C2), 136.7 (q, *J* = 3.3 Hz, C4), 134.6 (C7, C10), 130.2 (C8), 128.3 (C9), 123.8 (q, *J* = 270.0 Hz, CF_3), 121.7 (C-Cl), 121.2 (q, *J* = 33.0 Hz, C1), 49.1 (C*), 45.7 (C*), 42.0 (C*), 34.8 (C6), 33.9 (C5) [10].

*4.1.51 1-(4-Phenylpiperidin-1-yl)-4-(thiophen-2-yl)butan-1-one (**22**).*

Prepared from 4-phenylpiperidine (69 mg, 0.43 mmol) and 4-(thiophen-2-yl)butanoic acid (50 µL, 0.34 mmol) using HOBt (58 mg, 0.43 mmol), TBTU (140 mg, 0.43 mmol), NEt_3 (100 µL, 0.51 mmol), and DCM (6 mL) and following the general procedure described in method 2 section 4.1.7. The reaction mixture was stirred at room temperature for 18 h. DCM was removed under reduced pressure and the residue was purified using column chromatography (3:2, EtOAc:n-hexane) to give a clear oil, 105 mg (98%). R_f: 0.6 (3:2, EtOAc:*n*-hexane); IR (ATR): υ (cm^{-1}) 3026, 2933, 1636, 1435;^1H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.32 (m, *J* = 7.6 Hz, 2H), 7.25 – 7.16 (m, 3H), 7.13 (dd, *J* = 5.1, 1.2 Hz, 1H), 6.93 (dd, *J* = 5.1, 3.4 Hz, 1H), 6.89 – 6.78 (m, 1H), 4.82 (d, *J* = 13.2 Hz, 1H), 3.91 (d, *J* = 13.5 Hz, 1H), 3.09 (m, *J* = 13.2, 2.1 Hz, 1H), 2.94 (t, *J* = 7.4 Hz, 2H), 2.73 (m, *J* = 12.2, 3.6 Hz, 1H), 2.63 (m, *J* = 13.0, 2.1 Hz, 1H), 2.46 – 2.38 (m, 2H), 2.07 (m, *J* = 7.4 Hz, 2H), 1.93 – 1.84 (m, 2H), 1.67 – 1.54 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 170.8, 145.3, 144.6, 128.6, 126.8, 126.8, 126.5, 124.5, 123.2, 46.2, 42.8, 42.4, 34.0, 32.9, 32.3, 29.4, 27.2; HRMS calcd for $C_{19}H_{23}NOSNa$ $[M + H]^+$ 336.1393 found 336.1393.

*4.1.52 Methyl 4-(thiophen-2-yl)butanoate (**24**).*

4-(2-Thienyl)butyric acid (1 mL, 6.86 mmol) was dissolved in MeOH (10 mL) and concentrated H_2SO_4 (1.15 mL, 21.95 mmol) was added was added dropwise. The reaction mixture was stirred at room temperature for 22 h. MeOH was removed under reduced pressure and the residue dissolved in minimal H_2O. The pH was adjusted to pH = 7.0 using 2 M HCl and the aqueous layer was extracted with DCM (15 mL x 3). The combined organic layers were dried over $MgSO_4$ and the solvent removed under reduced pressure. The residue

was purified using column chromatography to give a clear oil, 1.03 g (81%). R_f: 0.7 (9:1, Pet. ether (40-60 ^oC):EtOAc); ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.09 (dd, *J* = 5.1 Hz, *J* = 1.2 Hz, 1H), 6.90-6.78 (m, 1H), 6.78-6.77 (m, 1H), 2.85 (t, *J* = 7.0 Hz, 2H), 3.65 (s, 3H), 2.35 (t, *J* = 7.0 Hz, 2H), 2.04-1.94 (m, 2H); ^1H NMR data matches literature data [37]; HRMS calcd for $C_9H_{13}O_2S$ $[M + H]^+$ 207.0450 found 207.0456.

4.2 NADH:ubiquinione oxidoreductase inhibition

Enzyme activity was examined in permeabilised mitochondria, isolated from rat liver, as previously described [10]. Basal activity of permeabilised mitochondria, incubated with 50 mM K_2HPO_4 pH 7.5, 3 mg/mL fatty acid free bovine serum albumin, 300 µM KCN and 100 µM NADH, was measured at 340 nm for 1 min. The oxidation of NADH was initiated with the addition of 60 µM ubiquinone and the resulting decrease in absorbance was followed for 3 minutes. Varying concentrations of test compounds were then added and absorbance was measured for a further 3 minutes. To determine the percentage activity of NADH:ubiquinone oxidoreductase, the slope of the line obtained with the addition of ubiquinone was divided by the slope of the line produced with the addition of the test compound. This value was then expressed as a percentage, relative to the value calculated for the vehicle control, DMSO, and the protein concentration of the mitochondrial sample. Specificity of the assay was confirmed with the addition of rotenone, a known inhibitor of NADH:ubiquinone oxidoreductase. Compounds were tested at 5 different concentrations, in triplicate, to generate IC_{50} values.

4.3 Cell culture and glucose uptake

C2C12 mouse muscle cells (Sigma-Aldrich, 91031101), were maintained in high glucose (4500 mg/ml) Dulbecco's Modified Eagle's Medium (DMEM) containing 100 µg/ml penicillin/streptomycin, 2 mM L-glutamine (complete medium) and 10 % (v/v) foetal bovine serum FBS. When 80 % confluent, differentiation into myotubes was induced with complete medium supplemented with 2 % (v/v) horse serum. Differentiated C2C12 were incubated with the compound of interest for 16 h in complete medium supplemented with 0.1 % (v/v) horse serum. Following stimulation, cells were washed with Krebs Ringer Buffer (KRB); 136 mM NaCl, 20 mM HEPES, 4.7 mM KCl, 1 mM $MgSO_4$, 1 mM $CaCl_2$, 4.05 mM Na_2HPO_4, 0.95 mM NaH_2PO_4, pH 7.4, warmed to 37 °C. Cells were then incubated with 1 µCi/mL [^3H]-2-deoxyglucose for 10 min at 37 °C. To terminate the assay, cells were washed three times with ice-cold KRB and lysed in 0.1 % (w/v) SDS for 30 min at 37 °C. Radioactivity of the lysates, diluted 1:4 in scintillation fluid, was determined with a 1450 Microbeta Liquid Scintillation Counter (PerkinElmer), expressing results as counts per minute (CPM). Results were normalised to the protein content of the sample using the bicinchoninic acid method [10, 21].

4.4 Molecular modelling

Of the 27 active compounds in the glucose update assay, 18 compounds had a p value < 0.001 significance; 3 had a P value < 0.01 and 6 at a P value of < 0.05. The remaining 19 compounds were not significant in the assay. Using Biovia Discovery Studio [24] Pareto

clustering was performed to generate a training (20% of compounds) and test (80% of compounds) data set for subsequent pharmacophore model generation and validation.

4.5 Pharmacophore model

For the pharmacophore model development the following compounds were assigned as most active with a principal number of 2 in Biovia Discovery Studio 4.0 (**3a** (RTC1), **9d, 16b, 16e**); as active with a principal number of 1 (**5c**, **3i**), and inactive with a principal number of 0 (**5a**, **3f**, **9a**).

3D conformations of the molecules were generated with the CAESAR algorithm and active molecules with *in vitro* activity were used to build a common features pharmacophore using uses the HipHop module of the Catalyst program. Descriptors such as hydrogen bond donor (HBD), hydrogen bond acceptor (HBA), hydrophobic (HY), ring aromatic (RA), positive ionizing (PI), and negative ionizing (NI) were utilised. The fitting method was set to rigid and the maximum omitted features to 1; a maximum of 50 exclusion volumes was allowed. With the result of the common feature pharmacophore model generation, desired chemical groups were identified using the feature mapping protocol. To make a prediction about the future performance of the developed pharmacophore model a ROCs curve was generated with a score of 0.712. The remaining compounds were used as a test set to validate the developed pharmacophore model. This test set was screened through the developed pharmacophore model to access its ability to distinguish active from inactive compounds.

4.6 In Silico pharmacokinetics

In silico absorption, distribution, metabolism, excretion and toxicity (ADMET) studies were performed using Biovia Discovery Studio 4.0. The module uses six mathematical models, to quantitatively predict properties by a set of rules/keys that specify threshold ADMET characteristics for the chemical structure of the molecules. These include predictive models for human intestinal absorption (HIA) [25, 27], aqueous solubility [26], blood brain barrier penetration, cytochrome P450 2D6 enzyme inhibition [28], plasma protein binding [29], and hepatotoxicity [30].

4.7 In vitro pharmacokinetics

Pharmacokinetic analysis was carried out as a service by Pharmidex, London, UK. See supporting information.

4.8 Thermodynamic solubility

Thermodynamic solubility was measured at concentrations of 1 mg/mL (2.61 mM, n =3) and 0.1 mg/mL (261 µM, n = 3). Compounds were equilibrated in 5 % (v/v) DMSO in PBS. Thermodynamic solubility was measured at 21 °C for 24 h. Samples were centrifuged at 15,000 *g* for 10 min and the supernatant was assayed by LC-MS/MS. See supporting information.

4.9 P-glycoprotein (P-gp) mediated efflux

All compounds were dissolved in 100% DMSO to provide 10 mM stock solutions from which donor (dose) solutions were prepared in DMEM to give a final drug concentration of 10 µM. All dose solutions contained 10 µM propranolol as an internal standard.

hMDR1-MDCK seeded filters were exposed to a fixed volume of the donor solution containing the compound of interest and its ability to traverse the monolayer and appear in the receiver compartment measured over a 30 minute period. Bidirectional permeability measurements were derived by examining the transfer of compound in both the apical to basolateral compartment, and vice versa. Sample analysis was conducted using LC-MS/MS with the detection settings optimised for each test compound, (n = 3). See supporting information.

4.10 Hepatocyte and microsomal stability

Buffer containing **3a** (RTC1) (1 µM) was added to a microtitre plate followed by hepatocytes. The reaction was incubated at 37 °C for up to 120 min. Acetonitrile, with an internal standard, was added to stop the incubation. Samples were centrifuged and the supernatant was then analysed by HPLC-MS/MS for the parent compound, n = 2. See supporting information.

4.11 Liver microsomal cytochrome analysis

CYP baculosomes containing cDNA for a single human P450 isozyme were added to a microtitre plate containing 10 µM **3a** (RTC-1), followed by the fluorogenic substrate and NADPH. The reaction was incubated at 37 °C, for at least 60 min, before fluorescence was measured, n = 3. See supporting information.

4.12 Plasma stability

Buffer containing 1 µM **3a** (RTC-1) was added to a microtitre plate followed by blood plasma. The reaction was incubated at 37 °C for 60 – 120 min. Acetonitrile, with an internal standard, was added to stop the reaction. Samples were centrifuged, and the supernatant was analysed by HPLC-MS/MS for the parent compound. See supporting information.

5. Acknowledgements

The authors would like to thank Maynooth University, Government of Ireland Postgraduate Scholarship from the Irish Research Council, Higher Education Authority PRTLI Cycle 5 BioAT Programme, and Science Foundation Ireland TIDA grant number 08/IN.1/B1900 for funding. We thank software vendor Biovia for their continuing support for academic research efforts. We also thank Dr Denise Rooney and Dr Diego Montagner for managing the NMR facility, Austin Power and Dr Joanne Masterson for their helpful discussion with regard to the glucose uptake assay work, and Dr Robert Elmes for managing the MS-HPLC facility (Science Foundation Ireland infrastructure grants 16/RI/3399 and 12/RI/2346/SOF).

6. References

1. International Diabetes Federation atlas 8th edition 2017.

2. N.C.D.R.F. Collaboration. Worldwide trends in diabetes since 1980: a pooled analysis of 751 population-based studies with 4.4 million participants, Lancet 387 (2016) 1513-1530.
3. Y. Zheng, S.H. Ley, F.B. Hu. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications, Nat. Rev. Endocrinol. 14 (2018) 88-98.
4. C. Hu, W. Jia. Therapeutic medications against diabetes: what we have and what we expect, Adv. Drug Deliv. Rev. 139 (2019) 3-15.
5. P.T. Donnan, T.M. MacDonald, A.D. Morris. Adherence to prescribed oral hypoglycaemic medication in a population of patients with Type 2 diabetes: A retrospective cohort study, Diabetic Medicine 19 (2002) 279–284.
6. H. Florez, J. Luo, S. Castillo-Florez, G. Mitsi, J. Hanna, L. Tamariz, A. Palacio, S. Nagendran, M. Hagan. Impact of metformin induced gastrointestinal symptoms on quality of life and adherence in patients with type 2 diabetes, Postgrad. Med. J 122 (2010) 112–120.
7. D. Kirpichnikov, S.I. McFarlane, J.R. Sowers. Metformin: An update, Annals of Internal Medicine 137 (2002) 25–33.
8. J. Wu, X. Luo, N. Thangthaeng, N. Sumien, Z. Chen, M. A. Rutledge, S. Jing, M. J. Forster, L. J. Yan. Pancreatic mitochondrial complex I exhibits aberrant hyperactivity in diabetes, Biochemistry and Biophysics Reports 11, (2017), 119–129.
9. W.L. Hou, J. Yin, M. Alimujiang, X.Y. Yu, L.G. Ai, Y. Bao, F. Liu, W.P. Jia. Inhibition of mitochondrial complex I improves glucose metabolism independently of AMPK activation, J. Cell. Mol. Med. 22 (2018) 1316-1328.
10. D.S.D. Martin, S. Leonard, R. Devine, C. Redondo, G.K. Kinsella, C.J. Breen, V. McEneaney, M.F. Rooney, T.S. Munsey, R.K. Porter, A. Sivaprasadarao, J.C. Stephens, J.B.C. Findlay. Novel mitochondrial complex I inhibitors restore glucose-handling abilities of high-fat fed mice, Journal of Molecular Endocrinology 56 (2016) 261–271.
11. R. Devine, D.S.D. Martin, G.K. Kinsella, J.B.C. Findlay, J.C. Stephens. Characterization of an aryl piperazine/2-hydroxypropyl-β-cyclodextrin association, a complex with antidiabetic potential, Results in Chemistry 2 (2020) 100026.
12. D.G. Hardie, B.E. Schaffer, A. Brunet. AMPK: An energy-sensing pathway with multiple inputs and outputs, Trends Cell Biol. 26 (2016) 190–201.
13. S. Leonard, L.M. Tobin, J.B.C. Findlay. The signalling mechanisms of a novel mitochondrial complex I inhibitor prevent lipid accumulation and attenuate TNF-α-induced insulin resistance in vitro, European Journal of Pharmacology 800 (2017) 1–8.
14. J. Yun, C. Rago, I. Cheong, R. Pagliarini, P. Angenendt, H. Rajagopalan, K. Schmidt, J.K. Willson, S. Markowitz, S. Zhou, L.A. Diaz Jr, V.E. Velculescu, C. Lengauer, K.W. Kinzler, B. Vogelstein, N. Papadopoulos. Glucose deprivation contributes to the development of KRAS pathway mutations in tumor cells, Science 325 (2009) 1555–1559.
15. H.L. Yale. The trifluoromethyl group in medicinal chemistry. J. Med. Chem. 1 (1959) 21–133.
16. C. Pinhong, L. Guosheng. Recent advances in transition-metal-catalyzed trifluoromethylation and related transformations, Synthesis 45 (2013) 2919-2939.
17. Applied Biophysics for Drug Discovery Editor(s): D. Huddler, E.R. Zartler, (2017) John Wiley & Sons Ltd. Chapter 2, Thermodynamics in Drug Discovery, R. O'Brien, N. Markova, G.A. Holdgate, Pages 7-28.

18. J. Åqvist, M. Kazemi, G. V. Isaksen, B.O. Brandsdal. Entropy and enzyme catalysis, Acc. Chem. Res. 50 (2017) 199−207.

19. K.D. Greis, S. Zhou, R. Siehnel, C. Klanke. Development and validation of a whole-cell inhibition assay for bacterial methionine aminopeptidase by surface-enhanced laser desorption ionization-time of flight mass spectrometry, Antimicrob. Agents Chemother. 49 (2005) 3428–3434.

20. Hollenback, D., Bonham, L., Law, L., Rossnagle, E., Substrate specificity of lysophosphatidic acid acyltransferase β - evidence from membrane and whole cell assays. J. Lipid Res. 47 (2006) 593–604.

21. Hernandez, L., Kodali, S., Cully, D., Singh, S., Wang, J., A target-specific whole cell assay for antibacterial drug discovery, Protoc. Exch. (2006). doi:10.1038/nprot.2006.130.

22. König, J.; Müller, F.; Fromm, M. F, Transporters and Drug-Drug Interactions: Important Determinants of Drug Disposition and Effects, Pharmacol. Rev. 65 (2013) 944–966.

23. Smith, P. K.; Krohn, R. I.; Hermanson, G. T.; Mallia, A. K.; Gartner, F. H.; Provenzano, M. D.; Fujimoto, E. K.; Goeke, N. M.; Olson, B. J.; Klenk, D. C., Measurement of Protein Using Bicinchoninic Acid, Anal. Biochem. 150 (1985) 76–85.

24. Dassault Systèmes BIOVIA, Discovery Studio Modeling Environment, Release 4.0, San Diego: Dassault Systèmes, 2013.

25. W. J. Egan, K. M. Merz, and J. J. Baldwin, Prediction of drug absorption using multivariate statistics, J. Med. Chem. 43 (2000) 3867–3877

26. A. Cheng and K. M. Merz, Prediction of aqueous solubility of a diverse set of compounds using quantitative structure-property relationships, J. Med. Chem. 46 (2003) 3572–3580.

27. W. J. Egan and G. Lauri, Prediction of intestinal permeability, Advanced Drug Delivery Reviews 54 (2002) 273–289.

28. S. L. Dixon and K. M. Merz, One-dimensional molecular representations and similarity calculations: methodology and validation, J. Med. Chem. 44 (2001) 3795–3809.

29. R. G. Susnow and S. L. Dixon, Use of robust classification techniques for the prediction of human cytochrome P450 2D6 inhibition, Journal of Chemical Information and Computer Sciences 43 (2003) 1308–1315.

30. A. Cheng and S. L. Dixon, In silico models for the prediction of dose-dependent human hepatotoxicity, Journal of Computer-Aided Molecular Design, 17 (2003) 811–823.

31. Cauquil-Caubère, I. & Kamenka, J. M. New structures able to prevent the inhibition by hydroxyl radicals of glutamate transport in cultured astrocytes, Eur. J. Med. Chem. 33 (1998) 867–877.

32. Brenner, E., Schneider, R., Fort, Y. Nickel-catalysed selective N -arylation or N,N'-diarylation of secondary diamines, Tetrahedron 58 (2002) 6913–6924.

33. Anastasiadis, C.; Hogarth, G.; Wilton-Ely, J. D. E. T. Functionalised dithiocarbamate complexes: Complexes based on indoline, indole and substituted piperazine backbones – X-ray crystal structure of $[Ni(S_2CNC_3H_6C_6H_4)_2]$. Inorganica Chimica Acta 363 (2010) 3222–3228.

34. Emmanuel Pinard, Alexander Alanine, Daniela Alberati, Markus Bender, Edilio Borroni, Patrick Bourdeaux, Virginie Brom, Serge Burner, Holger Fischer, Dominik Hainzl,

Remy Halm, Nicole Hauser, Synese Jolidon, Judith Lengyel, Hans-Peter Marty, Thierry Meyer, Jean-Luc Moreau, Roland Mory, Robert Narquizian, Mathias Nettekoven, Roger D. Norcross, Bernd Puellmann, Philipp Schmid, Sebastien Schmitt, Henri Stalder, Roger Wermuth, Joseph G. Wettstein, and Daniel Zimmerli Selective GlyT1 inhibitors: Discovery of [4-(3-fluoro-5- trifluoromethylpyridin-2-yl)piperazin-1-yl][5-methanesulfonyl-2-((S)-2,2, 2-trifluoro-1-methylethoxy)phenyl]methanone (RG1678), a promising novel medicine to treat schizophrenia. J. Med. Chem. 53 (2010) 4603–4614.

35. Stephens, J.; Findlay, J.; Kinsella, G.; Martin, D.; Devine, R.; Velasco-Torrijos, T. Preparation of N-acyl-N'-phenylpiperazine derivatives as sRBP modulators for use in the treatment of diabetes and obesity, (2013) WO2013060860A1.

36. A. Afzali-Ardakani, T.L. Breen, C.R. Kagan. Synthesis of soluble derivatives of sexithiophene and their use as the semiconducting channels in thin-film field-effect transistors, (2004) US006825358B2.

37. Fang, W., Jiang, J., Xu, Y., Zhou, J. & Tu, T. Novel robust benzimidazolylidene palladium complexes: Synthesis, structure, and catalytic applications in amination of chloroarenes, Tetrahedron 69 (2013) 673–679.

38. Baloglu, E.; Ghosh, S.; Lobera, M.; Schmidt, D. (2011) WO2011/88187.

- Forty one aryl piperazines derivatives have been generated and tested *in vitro* for their ability to stimulate glucose uptake.
- Six of the compounds were reported to effectively stimulate glucose uptake *in vitro* and inhibit NADH:ubiquinone oxidoreductase.
- The hit compound **3a** (RTC1) remained the most efficacious with a 2.57 fold increase in glucose uptake compared to vehicle control and micromolar inhibition of NADH:ubiquinone oxidoreductase (IC_{50} = 27 µM).
- Preliminary i*n vitro* DMPK and *in vivo* PK studies suggested that **3a** (RTC1) would not provoke adverse drug-drug interactions, yet be readily metabolised, avoid rapid excretion, with a short half-life, and have good tissue distribution.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: