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약학석사학위논문

새로운 AIMP2-DX2 저해제의 발굴과 구조-활성 관계 연구를 통한 항암제 개발

Discovery and Structure-Activity Relatioship of Novel AIMP2-DX2 Inhibitors as Anti-cancer Agents

2017년 2월

서울대학교 대학원

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서 보 경

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이 논문을 약학석사학위논문으로 제출함

2017년 2월

서울대학교 대학원

약학과 약품제조화학전공

서 보 경

서보경의 약학석사학위논문을 인준함

2017년 2월

위 원 장 _____ (인)

부위원장 _____ (인)

위 원 _____ (인)

국문 초록

P38 로도 알려진 AIMP2(ARS interacting multi-functional protein2)는 Aminoacyl tRNA synthetase 의 보조단백질 중 하나로 MSC(Multi-tRNA Synthetase Complex)라는 복합체를 이루며 단백질 합성에 관여한다. 기본적으로 AIMP2 는 TGF-beta 에 의한 성장 억제 신호를 강화시키고 p53 의 유비퀴틴화을 억제하여 세포자멸사를 유도하는 TNF 를 매개하는 중요한 역할을 하여 암을 억제한다고 알려져 있다. AIMP2 는 엑손 1 번부터 4 번까지 모두 발현된 full length 로 존재하지만 스플라이싱 변이체로 엑손 2 번이 결여되어있는 AIMP2-DX2(ARS interacting multi-functional protein2-Exon2 deleted)는 AIMP2-Full length 와 상반된 작용을 하여 과 발현 될 경우 발암을 유발하게 되며 특히 폐암세포에서 과 발현 되어있다.

따라서 본연구는 AIMP2-Full length 는 저해하지 않고 AIMP2-DX2 만을 선택적으로 저해하는 화합물을 합성하였다. 실험실 in-house library 를 통해 얻은 선도 물질의 저해 선택성, 활성과 물성을 개선하기 위해 선도 물질의 구조를 세 부분으로 나누어 다양한 작용기 도입하였으며 합성 경로의 key step 으로 szuki coupling, aromatic nucleophilic substitution reaction, 그리고 microwave irradiation-mediated aromatic amination

reaction 을 이용해 합성 과정을 최적화 하였다. 합성된 유도체들의 AIMP2-Full length & AIMP2-DX2 luciferase 와 WI-26 & A549 cell line 에 대한 cytotoxicity 를 평가하여 SAR 을 진행하였다. 최종적으로 AIMP2 에 비해 AIMP2-DX2 에 그리고 정상세포 대비 암세포 저해에 뛰어난 선택성을 가지며 submicromolar 수준의 AIMP2-DX2 저해 활성과 in vitro 항암활성을 가지는 신규 저분자 화합물을 수종 개발하였다.

Keywords: AIMP2-DX2, 항암 활성, 폐암, 저분자 화합물

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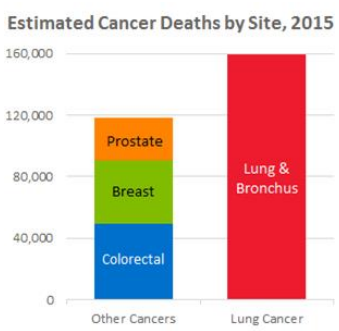
LIST OF ABBREVIATIONS

AIMP2	ARS Interacting Multi-Functional Protein2
AIMP2-DX2	ARS Interacting Multi-Functional Protein2-exon 2 deleted
Boc	Tert-butyloxycarbonyl
bs	Broad singlet
d	doublet
DCM	dichloromethane
DIPEA	*N,N'*-diisopropylethylamine
DME	dimethoxyethane
DMF	*N,N*-dimethyl formamide
EA, EtOAc	ethyl acetate
Et	ethyl
EtOH	ethanol
m	multiplet
MeCN	methyl cyanide
MeOH	methanol
MSC	Multi-tRNA Synthetase Complex
HEX	*n*-hexane
NMR	nuclear magnetic resonance
q	quartet
s	singlet
t	triplet
TFA	trifluoroacetic acid
TGF	transforming growth factor
THF	tetrahyrdofuran
TNF	tumor necrosis factor
UV	ultraviolet

I. 서론

1. 폐암치료제 개발의 필요성



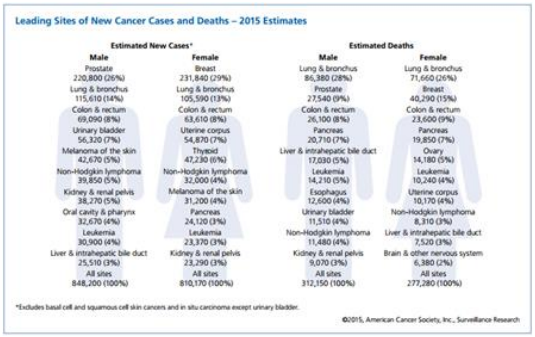


Figure 1. 2015년 미국 암 발병과 사망

2015 년도 미국의 통계에 따르면, 폐암의 발병률이 전체 암 중 2 위를 기록했으며 사망률은 1 위로 집계된다.[1] 국내에서도 폐암 발병률은 4 위로 나타났으며, 사망자 수는 1 위를 기록하였다. 또한 폐암에 의한 사망자수는 전립선암, 유방암, 대장암에 의한 사망자보다 많다고 한다. 전체 암의 일반적인 5 년 생존율이 약 60%정도인데 비해 폐암의 5 년 생존율은 약 20% 정도로 다른 암에 비해 치료가 어렵고 생존 가능성이 낮은 암으로 판단되므로 새로운 치료제의 개발이 시급한 실정이다.

폐암의 의료비용은 2014 년도 기준 미국에서만 131 억 달러 (약 16 조원)이며 이로 인한 생산성 손실은 361 억 달러 (약 44 조원)를 기록하였다. 전 세계 폐암 치료제 시장 규모는 2013 년 기준 51 억 달러를 상회했으며 2020 년도까지 79 억 달러로 증가할 것으로 예상된다.[2-6]

2. AIMP2-DX2 저해에 따른 암 억제 효과

P38 로도 알려진 AIMP2(ARS Interacting Multi-Functional Protein2)는 Aminoacyl tRNA synthetase 의 보조 단백질 중 하나로 MSC 라는 복합체를 이루며 정상상태에서 단백질 합성에 관여한다. 그 외에도 AIMP2 는 TGF-beta 에 의한 성장 억제 신호를 강화시키고 세포자멸사와 관련된 p53 및 TNF-alpha 를 매개하는 중요한 역할을 하며 암을 억제한다고 알려져 있다. RNA 가공이 일어나는 과정에서, 엑손이 1 번부터 4 번까지 모두 발현되면 full length 가 되고, 스플라이싱 변이체로 엑손 2 번이 결여되면 AIMP2-DX2 가 된다. 앞서 말씀드렸듯이 AIMP2 는 다양한 기전을 통해 암 억제 인자로 역할하지만, AIMP2-DX2 는 이러한 AIMP2 에 대해 길항제와 같은 역할을 하면서 항상성 균형을 이루고 있다. 그러나 두 단백질 간의 불균형은 암화 현상을 증가시킬

수 있으며, 실제로 암환자의 조직 세포에서 AIMP2-DX2 의 양이 늘어남을 관찰할 수 있다. 또한, AIMP2-DX2 를 억제시키면 암의 크기가 줄어드는 것을 실험으로 확인한 바 있다.[7] 이러한 암세포와의 상관성으로 AIMP2-DX2 저해제의 폐암 치료제로서의 가능성이 확인 되었고, AIMP2-DX2 만을 선택적으로 저해하는 화합물을 합성하고자 연구를 진행하였다.

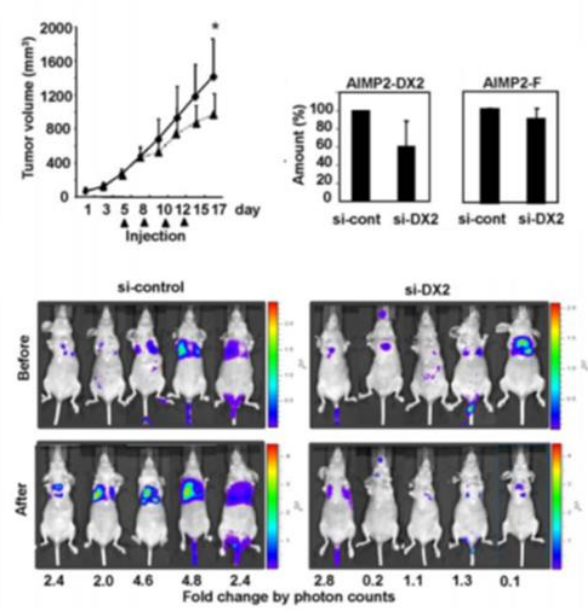


Figure 2. AIMP2-DX2를 knock-down 시킬 경우, 나타나는 암 억제 효과

3. AIMP2-DX2 저해 저분자 물질을 통한 항암 작용

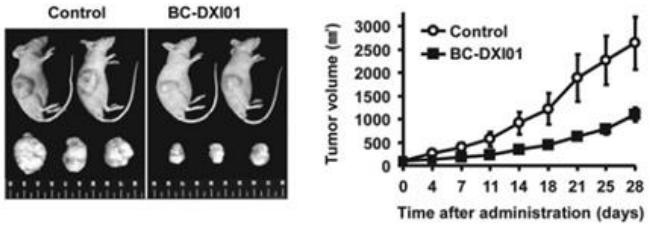


Figure 3. Mouse xenograft model에서 BC-DXI01의 항암 활성

이러한 AIMP2-DX2 를 저해하는 물질을 암 억제를 위한 치료제로 개발하기 위하여, in-house library 화합물의 AIMP2-DX2 억제 효과 및 암세포 사멸 효과를 확인하였다. 그 결과, AIMP2-DX2 발현 억제 효과를 보이는 동시에 유의미한 암세포 사멸 효과를 갖는 화합물을 도출하였으며, 동물 실험을 통하여 화합물의 종양 크기 억제 효과도 확인하였다. 개선된 활성을 갖는 화합물을 도출하기 위하여 유사 골격을 지닌 본 실험실 라이브러리 내의 화합물의 활성 탐색을 통하여 초기 화합물에 비해 AIMP2-DX2 억제 효과와 암세포 사멸 효과가 개선된 화합물 도출에 성공하였다.[8]

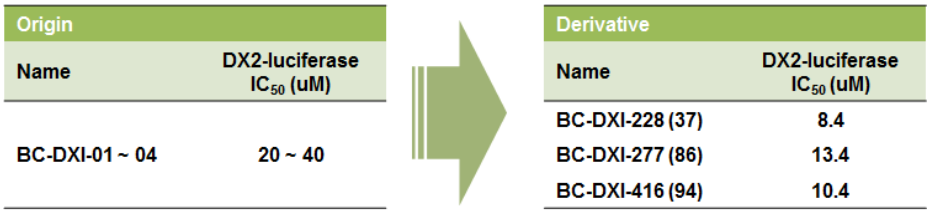


Figure 4. in-house library 검색을 통한 AIMP2-DX2 억제 효과 개선된 화합물 도출

새롭게 도출한 골격을 포함하는 본 실험실 라이브러리 내 화합물군의 활성 비교를 통하여 특정 위치의 치환기 변화에 따라 AIMP2-DX2 저해효과 및 암 세포 사멸 효과에 대한 대략적인 구조-활성 상관관계를 예측할 수 있었다. 따라서 AIMP2-DX2 저해 목적의 본격적인 구조 수식을 통하여 암세포 억제 활성을 증가시키면서, 더 나아가 용해도와 안정성이 개선된 최적화 화합물을 도출하여 새로운 저분자 약물을 제안하고자 한다. 또한 의약품 후보물질로서의 화합물의 가치를 확보하기 위하여 기존의 합성법을 개선하여 보다 효율적인 방법으로 목적 화합물을 합성하는 것을 부가적인 목표로 하였다.

4. 선행 연구

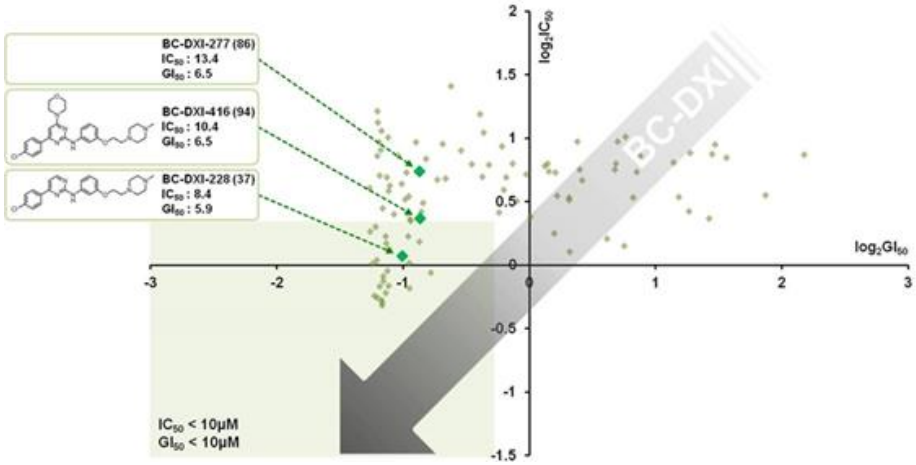


Figure 5. in-house library 검색을 통한 핵심 scaffold 도출

본 실험실이 구축한 Focused in-house library 에서 얻은 화합물들로 일차 스크리닝을 진행한 결과 SAP 37, SAP 86, SAP 94 등과 같이 활성이 개선된 화합물들을 수 종 도출하였다. 이후 fast in vivo study 를 통해 mouse 모델에서의 동물실험 결과에서 암세포 사멸을 촉진하는 효과를 확인하였다. 그러나 SAP 37 의 사례와 같이 약물을 주입한 국소적인 부분의 암세포만 사멸한 결과가 있어 해당 화합물의 용해도 등의 물리화학적 특성도 개선하는 것을 부가적인 목표로 하였다.

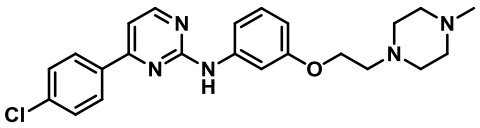


Figure 6. in-house library 검색을 통해 도출된 SAP 37 (1)의 구조

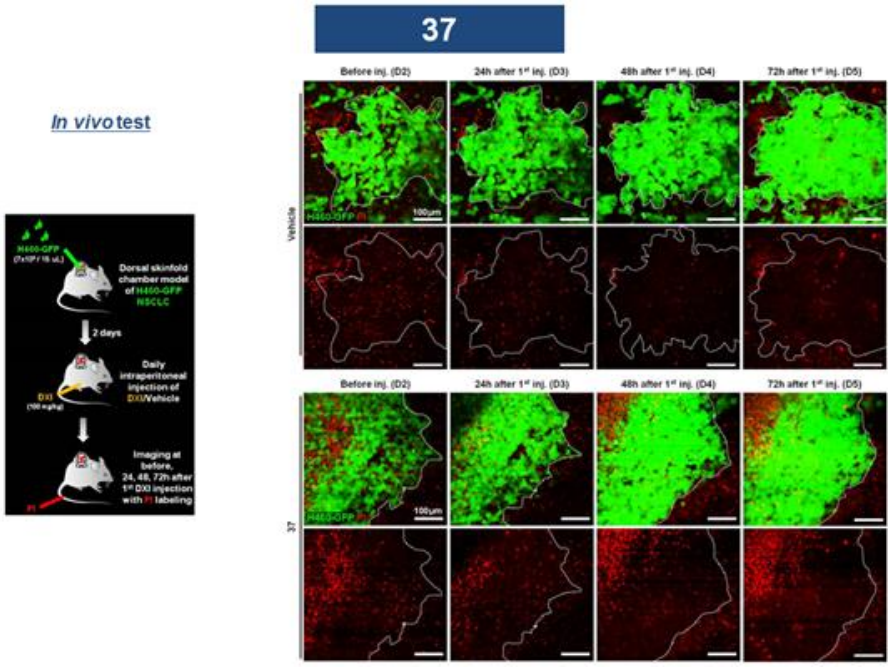


Figure 7. SAP 37의 fast *in vivo* test

이후 시행된 추가 화합물들의 스크리닝에서 선도 화합물인 SAP 160 을 도출하였다. SAP 160 은 10 μM 미만의 IC_{50} 값과 GI_{50} 값을 보였으며, 이는 현재까지 검색된 화합물 중 가장 뛰어난 결과로서, 기전적으로 억제하지 않아야 하는 정상 AIMP2 에 대한 AIMP2-DX2 저해의 선택성과 더불어, 암세포인

A549 세포주와 정상 세포인 WI-26 세포주 사이의 세포 성장 저해 선택성 또한 확인 할 수 있었다.

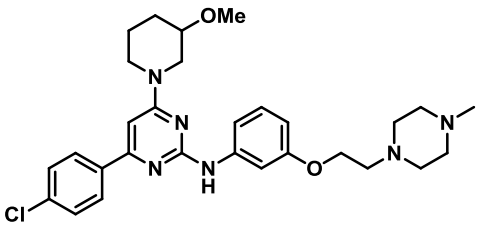


Figure 8. Lead 화합물로 도출된 SAP 160 (2)

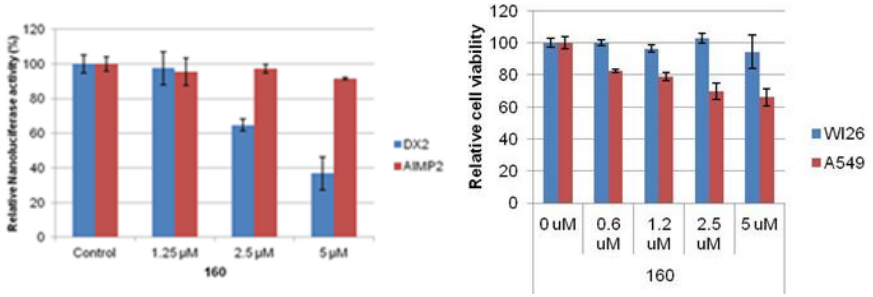


Figure 9. SAP 160 (2)의 Nano-Luciferase assay (왼쪽)과 MTT assay (오른쪽)

선도 화합물 SAP 160 은 잘 알려진 강력한 항암제인 Taxol 을 대조군으로 사용한 in vivo test 에서도 Taxol 치료 농도 처리 결과와 비견할만한 활성을 보였다. SAP160 과 Taxol 모두 암의 무게와 크기를 기대할만한 수준으로 줄였고, 쥐의 체중 변화는 거의 관찰되지 않아 간접적으로 안전성을 확보하였다.

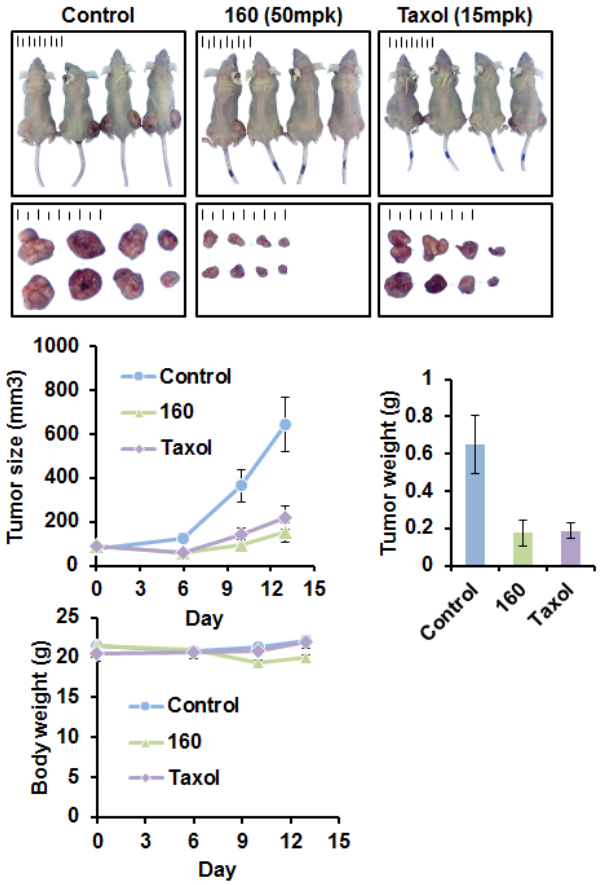


Figure 10. SAP 160 (2)과 Taxol의 *in vivo* test

SAP 160 을 선도 화합물로 하여 개선된 AIMP2-DX2 nano-luciferase 활성과 선택성, 그리고 MTT assay 에서 A549 세포주를 선택적으로 사멸시키는 화합물의 도출을 목표로 하였다. 또한 이러한 유도체들은 *in vivo* test 및 독성 시험을 거쳐 임상 적용의 가능성을 확보하기 위하여 용해도 등의 물성을 고려한 구조를 설계, 합성하였다.

II. 본론

1. 유도체 설계 전략

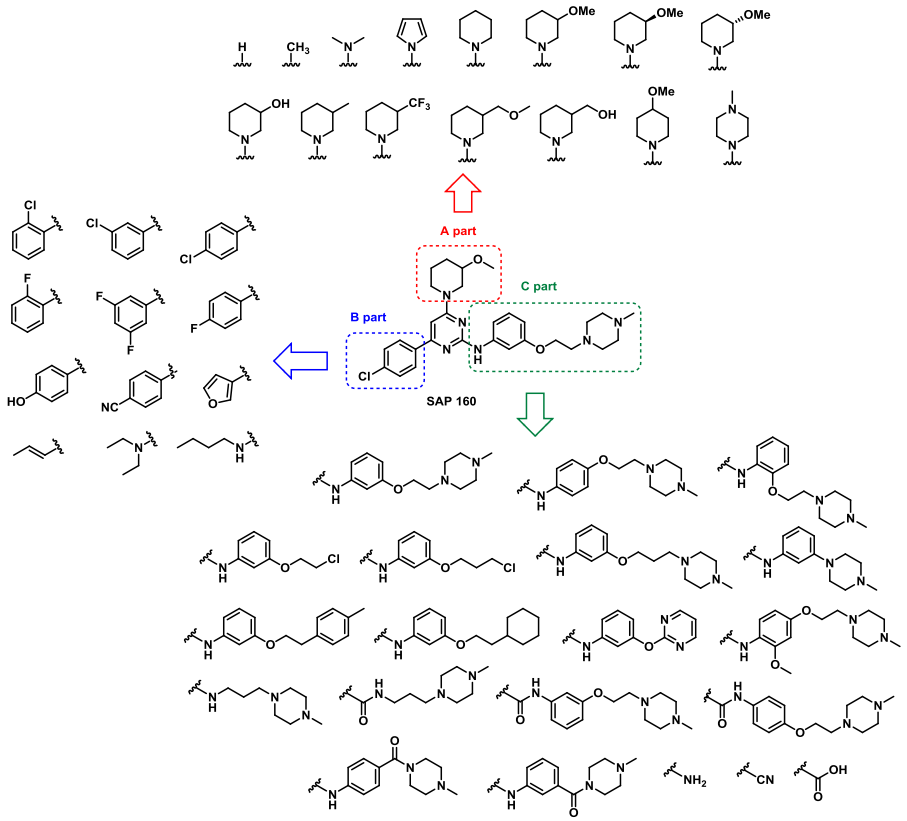


Figure 11. SAP 160 (2)의 구조적 분석 및 디자인

선도 화합물 SAP160 화합물로부터 다양한 관점에서 개선을 진행하고자 하였으며, 특히 재현성이 없고 AIMP2 대비 DX2에 대한 선택성이 사라진 어세이 결과를 얻어 여기에 초

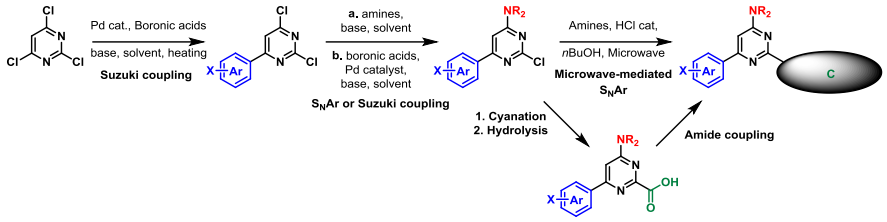
점을 맞춰 활성과 선택성을 개선하고자 진행하였다. 선도 화합물 SAP160의 구조를 그림 11에서와 같이 핵심 골격인 pyrimidine 고리를 중심으로 잔기를 3-methoxy piperazine part (A part), aryl part (B part), piperazine contained aniline part (C part)의 세 부분으로 나누어 기능기를 변화 시키는 전략을 통해 다양한 화합물의 합성을 진행하였다.

A part는 3-methoxy piperidine의 steric factor, 3-methoxy 작용기의 electronic factor 및 hydrogen bonding acceptor로의 작용을 확인하기 위해 여러가지 치환기를 도입하였다. B part의 경우 benzene ring에 chloride 치환기의 위치에 따른 활성 변화와 electron donating group인 hydroxy기 또는 electron withdrawing group인 nitrile기를 도입에 따른 활성 변화를 관찰하고자 하였다. 그 외에도 alkene, furan ring, 이차 아민, 삼차 아민과 같은 벤젠고리가 아닌 다른 치환기를 도입함으로써 벤젠고리의 역할을 확인하고자 하였다. C part는 aniline, alkoxy linker, methyl piperazine 세 부분으로 나누어 생각할 수 있고, 가장 먼저 alkoxy group의 위치를 바꿔 보았다. Alkoxy linker의 길이를 변형해 보기도 하고, methyl piperazine을 없애거나 benzene, cyclic hexane, pyrimidine으로 대체하기도 하였다. Aniline과 pyrimidine 골격 사이에 amide bond를 도입하거나, methyl piperazine과

aniline 사이에 carbonyl group을 도입하기도 하였다. 또한 물성적인 측면과 화학적 안정성, 그리고 hERG receptor와의 친화력 감소를 위해 C part의 단순화를 시도하였고 일차 아민, nitrile, carboxylic acid로 바꿔보았다.

2. 합성

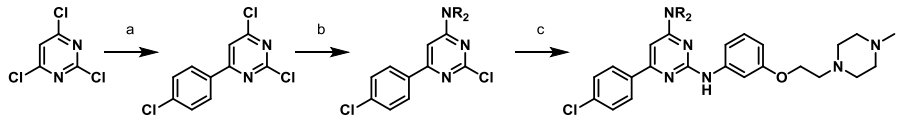
2,4,6-Trichloropyrimidine에 Suzuki coupling을 이용하여 aryl group을 도입하여 arylpyrimidine을 합성하였다. 혹은 base를 이용하여 이차 아민과 삼차 아민을 도입하여 aminopyrimidine을 합성하였다. 여기에 nucleophilic aromatic substitution을 통해 aliphatic amine을 도입하여 arylamine을 합성하였다. 여기서 microwave를 이용하여 nucleophilic aromatic substitution 해주어 최종화합물을 얻거나, cyanide 도입 후 가수분해하여서 acid를 얻었다. 이 acid를 dichloromethane 용매에서 DMF 촉매 하에 oxalyl chloride를 이용하여 acid halide로 만든 뒤 aniline 혹은 amine과 축합하여 최종화합물을 얻을 수 있었다. (**Scheme 1**)



Scheme 1. General scheme of pyrimidine analogues

2. 1. A part modification

2,4,6-Trichloridepyrimidine **3** 에 palladium 촉매로 Suzuki coupling 을 이용하여 4-chlorophenyl group 을 도입하여 4-chlorophenylpyrimidine **4** 을 합성하였다. 여기에 nucleophilic aromatic substitution 을 통해 aliphatic amine 을 도입하여 arylamine **5a–5l** 을 합성하였다. 여기서 microwave 를 이용하여 nucleophilic aromatic substitution 해주어 최종화합물 **2**, **6a–6l** 을 얻었다.（**Scheme 2**）

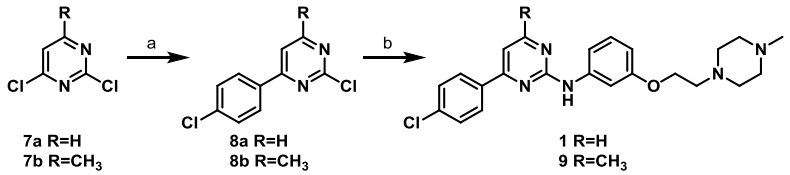


Scheme 2. Scheme for A part modification: Amines

Reagents and conditions: (a) 4-chlorophenylboronic acid, $Pd(PPh_3)_4$, Na_2C

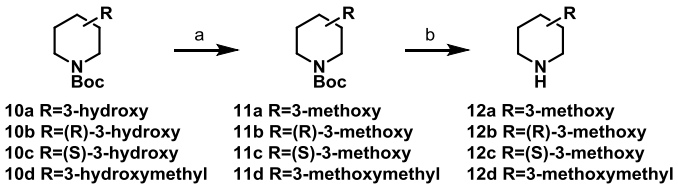
O_3, H_2O, ethylene glycol dimethyl ether, reflux, 27%; (b) Amines, *i*Pr_2Et N, EtOH, 27–78%; (c) HCl, BuOH, microwave, 3–22%.

2,4-dichloropyrimidine **7a** 혹은 2,4-dichloro-6-methylpyrimidine **7b** 에 palladium 촉매로 Suzuki coupling 을 이용하여 4-chlorophenyl group 을 도입하여 4-chlorophenylpyrimidine **8a**, **8b** 을 합성하였다. 여기에 microwave 를 이용하여 nucleophilic aromatic substitution 해주어 최종화합물 **1**, **9** 을 얻었다. (**Scheme 3**)



Scheme 3. Scheme for A part modification: proton and methyl group
Reagents and conditions: (a) 4-chlorophenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, H_2O, ethylene glycol dimethyl ether, reflux, 72–74%; (b) HCl, BuOH, microwave irradiation, 17–26%.

상업적으로 구매 가능하지 않은 piperidine 유도체의 경우 다음과 같은 방법으로 합성하였다. Boc protection 되어있으며 hydroxyl 기를 가지고 있는 piperidine **10a**–**10d** 에 methylation 해주어 methoxy group 을 도입하여 **11a**–**11d** 를 합성하였다. Boc deprotection 을 해주어 piperidine 유도체 **12a**–**12d** 를 합성하였다. (**Scheme 4**)

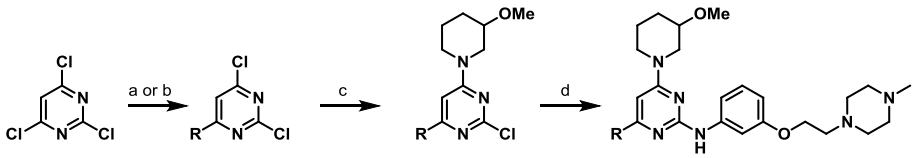


Scheme 4. Synthesis of piperidine analogues

Reagents and conditions: (a) NaH, MeI, THF, 83–87%; (b) 10% TFA in CH_2Cl_2.

2. 2. B part modification

2,4,6-Trichloridepyrimidine 에 palladium 촉매로 Suzuki coupling 을 이용하여 aryl group 을 도입하여 arylpyrimidine 을 합성하였다. 혹은 base 를 이용하여 이차 아민과 삼차 아민을 도입하여 aminopyrimidine 을 합성하였다. 여기에 nucleophilic aromatic substitution 을 통해 3-methoxypiperidine 을 도입하여 arylamine 을 합성하였다. 여기서 microwave 를 이용하여 nucleophilic aromatic substitution 해주어 최종화합물을 얻었다. (**Scheme 5**)

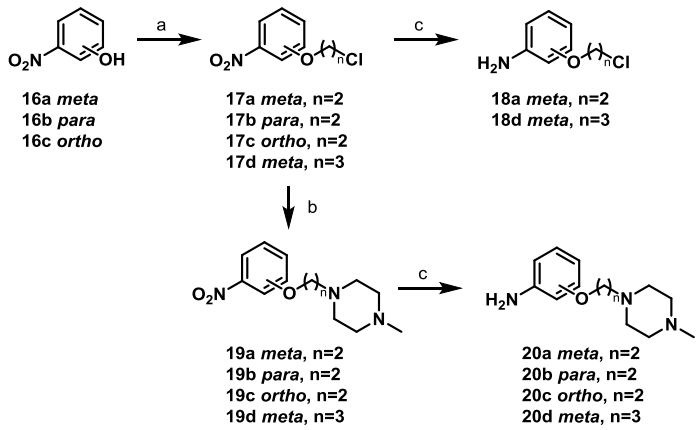


Scheme 5. Scheme for B part modification

Reagents and conditions: (a) $Pd(PPh_3)_4$, Na_2CO_3, H_2O, THF, reflux, 32–76%; (b) *i*Pr_2EtN, Ethanol, 46–64%; (c) 3-methoxypiperidine, *i*Pr_2EtN, EtOH, 48-64%; (d) HCl, BuOH, microwave, 5–10%.

2. 3. C part modification

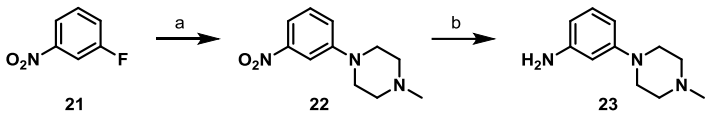
상업적으로 구매 가능한 nitrophenol 에 2-chloroethanol 또는 3-chloropropanol 과 Mitsunobu reaction 을 하여 regiosiomers **17a–17d** 를 합성하였다. $ZnCl_2 \cdot 2H_2O$ 로 환원시켜 **18a**, **18d** 를 합성하거나, 4-methylpiperazine 과 nucleophilic substitution 한 **19a–19d** 를 환원시켜주어서 aniline **20a–20d** 을 얻고, 이후의 coupling 에 사용하였다. (**Scheme 6**)



Scheme 6. Scheme for alkoxy linker of C part modification

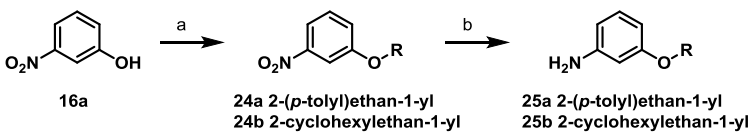
Reagents and conditions: (a) 2-chloroethanol, PPh_3, DIAD, THF, 63–84%; (b) 1-methyl piperazine, K_2CO_3, TBAI, DMF, 46–57% (c) $SnCl_2 \cdot 2H_2O$, EtOH, 26–90%.

Alkoxy linker 가 없을 때 활성 차이를 보기 위해 상업적으로 구매 가능한 1-fluoro-3-nitrobenzene **21** 에 1-methylpiperazine 을 치환시켜주고, nitro group 을 reduction 시켜주어 **23** 을 합성하였다. (**Scheme 7**)



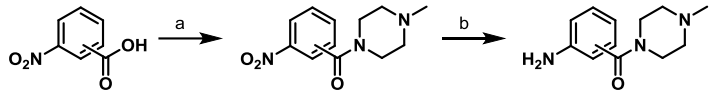
Scheme 7. Scheme for C part modification: deletion of alkoxy linker
Reagents and conditions: (a) 1-methyl piperazine, K_2CO_3, DMSO, 18%; (b) Pd/C, H_2, MeOH, 91%.

1-Methylpiperazine 의 효과를 확인하기 위하여 methylphenyl group 과 cyclohexyl 을 도입해 보았고 다음과 같은 방법으로 합성하였다. 상업적으로 구매가능한 3-nitrophenol **16a** 에 Mitsunobu reaction 을 이용하여 **24a**, **24b** 를 합성하였다. Nitro group 을 환원시켜주어 **25a**, **25b** 를 합성하였다. (**Scheme 8)**



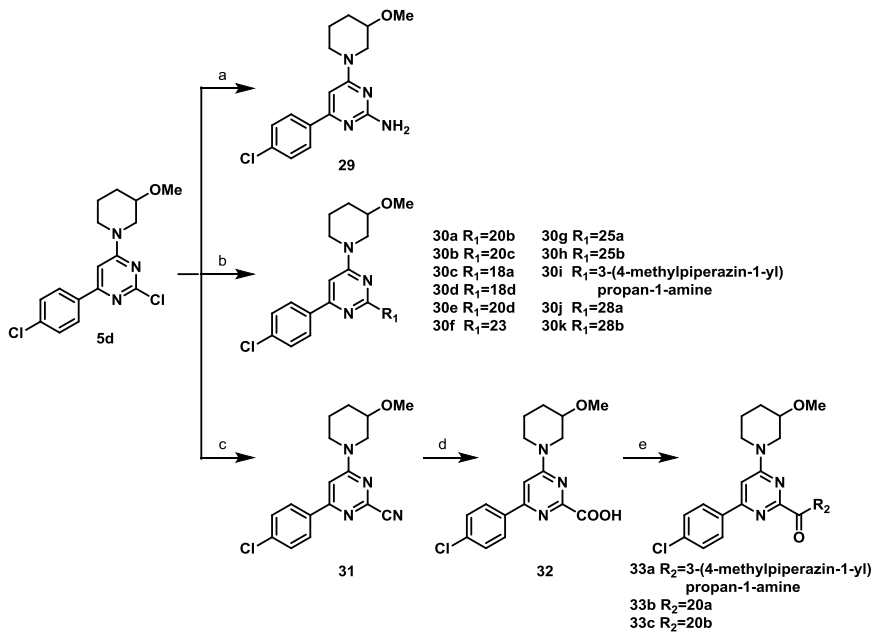
Scheme 8. Scheme for C part modification: piperazine group
Reagents and conditions: (a) PPh_3, DIAD, THF, 59–67%; (b) $SnCl_2{\cdot}2H_2O$, EtOH, 40–50%.

Alkoxy group 을 carbonyl group 으로 바꿔보기 위해 다음과 같은 방법으로 합성하였다. 상업적으로 구매 가능한 nitrobenzoic acid **26a**, **26b** 에 1-methylpiperazine 을 amide coupling 을 통해 도입하여 **27a**, **27b** 를 합성하였다. Palladium 을 이용하여 nitro group 을 환원시켜주어 **28a**, **28b** 를 합성하였다.(**Scheme 9**)



Scheme 9. Scheme for alkoxy linker of C part modification
Reagents and conditions: (a) (i) oxalyl chloride, DMF, CH_2Cl_2, rt (ii) 1-methyl piperazine, THF, rt , 79–81% for 2 steps; (b) Pd/C, H_2, MeOH, rt, 92–93%.

4-chlorophenyl 과 3-methoxypiperidine 치환되어있는 pyrimidine **5d** 에 amine 을 도입하여 **29** 를 합성하거나, 위와 같은 방법으로 합성하거나 상업적으로 구매 가능한 여러가지 aniline 및 amine analagoue 들을 HCl 촉매 하에 microwave irradiation 하여 **30a**–**30k** 를 합성할 수 있었다. 또는 **5d** 에 cyanide 를 도입한 **31** 을 가수분해하여 **3**2 를 얻은 후 여러 가지 aniline 혹은 amine analogue 와 amide coupling 해주어 **33a**–**33c** 를 합성하였다. (**Scheme 10**)



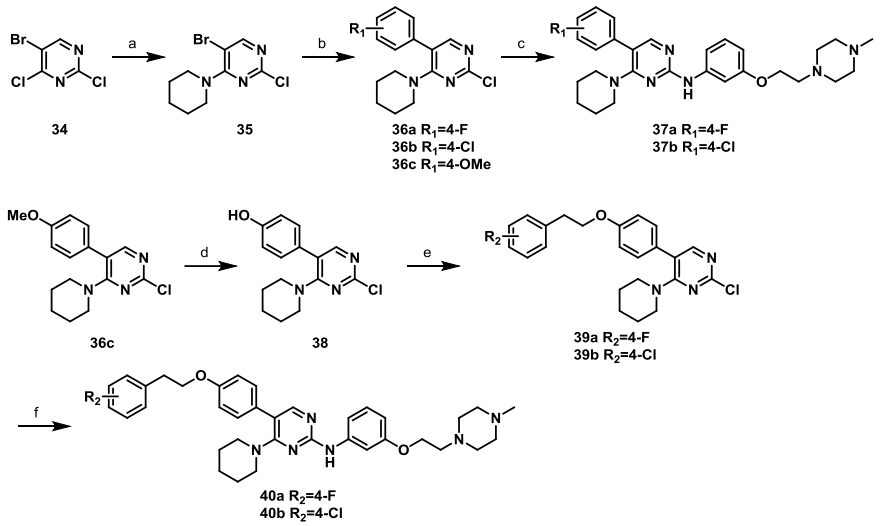
Scheme 10. Scheme for C part modification and syntheses of final compounds

Reagents and conditions: (a) aq.NH_4OH solution, 1,4-dioxane, micrrowave irradiation, 40%; (b) aniline or amine analogues, HCl, BuOH, microwave, 7–19%; (c) NaCN, DABCO, DMSO, 69%; (d) KOH, 50% aq.EtOH, 60%; (e) (i) oxalyl chloride, DMF, CH_2Cl_2, rt (ii) 1-methyl piperazine, THF, rt, 31–41% for 2 steps.

2. 4. Position exchange

상업적으로 구매 가능한 5-bromo-2,4-dichloropyrimidine **34** 에 piperidine 을 치환시켜 **35** 를 합성하였다. 여기에 Suzuki coupling 을 이용하여 다양한 phenyl group 을 도입하여 **36a–36b** 를 합성하였다. 여기에 바로 aniline **20a** 와 coupling

해주어 **37a**, **37b** 를 합성하였다. Methoxy 가 들어있는 **36c** 의 경우 BBr_3 를 이용하여 demethylation 후 Mitsunobu reaction 을 통해 **39a**, **39b** 를 합성한 후 aniline **20a** 와 coupling 해주어 **40a**, **40b** 를 합성하였다. (**Scheme 11**)

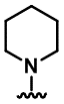
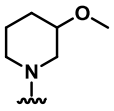
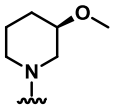
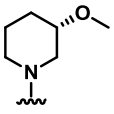


Scheme 11. Scheme for Position exchanged analogues

Reagents and conditions: (a) Piperidine, TEA, EtOH, 83%; (b) Phenylboronic acid, Na_2CO_3, $Pd(PPh_3)_4$, H_2O, DME, reflux, 66–87%; (c) HCl, BuOH, microwave, 41–43%; (d) BBr_3, CH_2Cl_2, 68%; (e) Phenylethanol, PPh_3, DIAD, THF, 93–99%; (f) HCl, BuOH, microwave, 32–35%.

3. 활성 평가

3. 1. *In vitro* DX2 nanoluciferase activity results of A part modified analogues

Analogues	Structure	% Inhibition at 5μM (S.D.)
1		31.9 (28.1)
9		19.3 (17.9)
6a		9.9 (13.4)
6b		26.8 (17.4)
6c		69.68 (9.57)
2		80.48 (1.98)
6e		44.38 (8.64)
6f		56.32 (2.16)

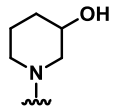
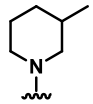
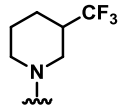
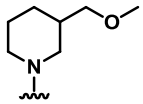
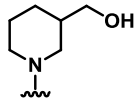
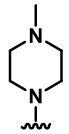
6g		34.30 (1.75)
6h		6.83 (13.68)
6i		40.41 (6.04)
6j		21.75 (9.41)
6k		4.78 (5.97)
6l		62.88 (1.39)

Table 1. *In vitro* DX2 nanoluciferase activity results of A part modified analogues

수소 대신 메틸기를 도입하였을 때 DX2 저해 활성이 감소하였고, 큰 alkyl group 을 도입하기에는 물성이 좋지 않아질 것이라 예상되어 tertiary amine group 으로 대체하였다. Tertiary amine group 을 갖는 유도체들의 경우 tertiary amine group 의 크기가 커질수록 DX2 저해 활성이 증가하였다. 따라서 A part 는 steric factor 가 중요하게 작용한다는 것을 알

수 있었다. SAP 160 의 A part 인 3-Methoxy piperidine 의 3-methoxy group 의 역할과 electronic factor 를 확인하기 위해 다양한 치환기로 바꿔보았다. 3-Methoxy group 이 치환되어 있는 chiral center 에 대해 chiral switch 전략을 취했을 때, R form 과 S form 에서 유사한 활성을 나타내어 광학 활성에 대한 활성 차이는 보이지 않는 것을 확인할 수 있었다. 또, piperidine 의 3-methoxy group 을 hydroxyl group, methyl group 으로 바구거나 trifluoromethyl group 으로 바꿔도 활성이 떨어짐을 확인할 수 있었다. 이를 통해 3-methoxy group 이 hydrogen-bonding acceptor 역할을 한다고 추측할 수 있었다. 3-Methoxy group 의 산소원자의 위치가 이동된 3-methoxymethyl, 3-hydroxymethyl, 4-methoxy group 을 갖는 화합물들의 활성이 감소된 것으로 보아 size-limited hydrogen-bonding 을 한다고 생각할 수 있었다. 또한, 4-methyl piperazine 으로 대체하였을 때 62.88% 저해하여 나쁘지 않은 활성을 가져 hydrogen bonding acceptor 가 piperidine 혹은 piperazine 의 ring 과 가까울수록 좋다는 것을 알 수 있었다.

3. 2. *In vitro* DX2 nanoluciferase activity results of B part modified analogues

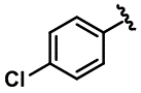
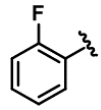
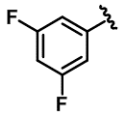
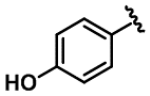
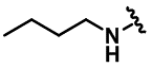
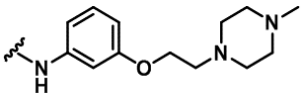
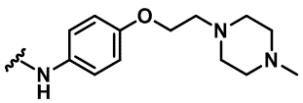
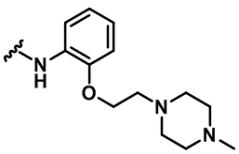
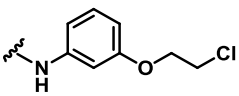
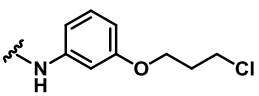
Analogues	Structure	DX2 Luciferase Activity % Inhibition at 5μM (S.D.)
2		80.48 (1.98)
15a		45.16 (3.11)
15b		55.82 (12.68)
15c		36.07 (7.96)
15d		19.11 (6.24)
15e		15.73 (4.94)
15f		6.54 (12.38)

Table 2. *In vitro* DX2 nanoluciferase activity results of B part modified analogues

B part 는 benzene ring 의 substituent 에 따른 effect 와 benzene ring 자체의 역할을 알아 보기 위한 유도체들을

합성하고 평가하였다. 치환기 자체 혹은 치환기의 위치를 바꾸거나 benzene ring 을 없애고 alkene 과 tertiary amine, secondary amine 을 도입해 보았지만 활성의 개선은 없었다.

3. 3. *In vitro* DX2 nanoluciferase activity results of C part modified analogues

Analogues	Structure	DX2 Luciferase Activity % Inhibition at 5μM (S.D.)
2		80.48 (1.98)
30a		14.95 (6.53)
30b		59.38 (2.88)
30c		14.08 (5.83)
30d		31.48 (3.38)

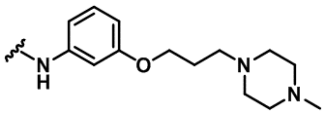
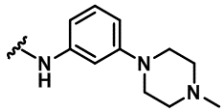
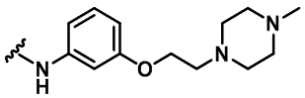
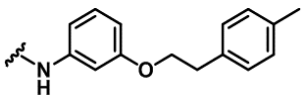
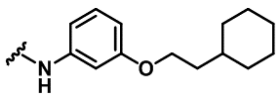
30e		44.48 (2.47)
30f		32.92 (9.61)

Table 3. *In vitro* DX2 nanoluciferase activity results of alkoxy linker of C part modified analogues

C part 는 aniline, alkoxy linker, N-methyl piperazine 세 부분으로 나누어 생각할 수 있다. 먼저 alkoxy group 의 위치를 바꿔보았고, N-methyl piperazine 을 없애거나, linker 길이를 변형했을 때 활성이 감소하는 것을 확인하였다.

Analogues	Structure	% Inhibition at 5μM (S.D.)	
		A549 cell viability	WI-26 cell viability
2		98.93 (0.48)	96.76 (0.67)
30g		24.07 (12.63)	10.14 (5.47)
30h		33.06 (8.98)	8.4 (3.25)

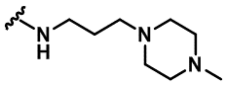
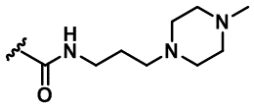
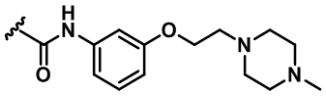
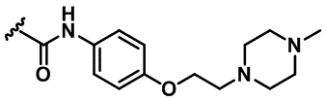
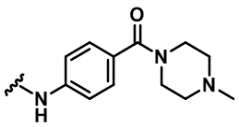
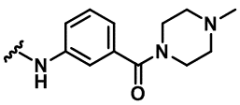
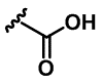
30i		42.73 (3.06)	10.37 (1.48)
33a		29.89 (9.3)	10.2 (5.22)
33b		78.15 (0.83)	82.2 (0.54)
33c		87.5 (0.57)	67.92 (0.44)
30j		68.26 (0.88)	1.7 (5.67)
30k		68.65 (2.86)	5.79 (3.95)
29		62.11 (1.98)	54.59 (0.6)
31		64.62 (4.11)	12.51 (5.22)
32		5.09 (3.93)	26.03 (2.1)

Table 4. *In vitro* DX2 nanoluciferase activity results of benzene ring and piperazine of C part modified analogues

N-methyl piperazine 을 benzene, cyclic hexane, pyrimidine 으로 대체하거나, 새로운 functional group 인 methyl 을 도입하였을

때 모두 A-549 cell 에 대한 독성이 감소하였다. Benzene ring 을 없애고 aliphatic chain 을 도입하거나, 다양한 aniline analogues 의 C part 를 amide bond 로 연결해보기도 했지만 A-549 cell 에 대한 독성이 감소하였다. Benzene ring 과 N-methyl piperazine 사이의 alkoxy linker 를 carbonyl 로 바꾸었을 때에도 활성적 측면에서의 개선은 일어나지 않았다.

이후에 물성적인 측면과 화학적 안정성, 그리고 hERG receptor 와의 친화력 감소를 위해 C part 를 단순화하였다. 이 중 simple amine 과 nitrile 기가 안정적이며 일정 수준 이상의 활성을 가졌고, carboxylic acid 기의 경우 활성을 잃었다.

3．4．*In vitro* DX2 nanoluciferase activity results of position exchanged analogues

Analogues	% Inhibition (S.D.)	
	A549 cell viability at 5μM	WI-26 cell viability at 5μM
37a	87.45 (0.99)	15.92 (6.62)
	H460 cell viability at 10μM	WI-26 cell viability at 10μM
37b	98.62 (0.28)	18.58 (10.23)

40a	97.06 (3.46)	41.73 (3.44)
40b	88.34 (5.48)	5.98 (6.77)

Table 5. *In vitro* DX2 nanoluciferase activity results of position exchanged analogues

다음 세대 유도체로 pyrimidine 골격의 치환체 위치를 바꿔주는 화합물들을 설계 및 합성하였고, 좋은 항암 활성을 보이는 유도체들을 얻을 수 있었다.

3. 5. 투여량 의존적 반응과 선택성

Analogues	IC50 (Luciferase assay, μM)		GI50 (MTT assay, μM)	
	DX2	**AIMP2**	**A549**	**WI-26**
2	**3.59**	**-**	**2.77**	**-**
6l	**10.69**	**ND**	**3.4**	**>100**
15a	**5.039**	**ND**	**7.777**	**>100**
30j	**3.281**	**5.681**	**15.85**	**ND**
31	**2.709**	**7.708**	**0.9849**	**ND**
37a	**1.118**	**4.132**	**0.8588**	**ND**

Table 6. Dose-dependent response and selectivity of selected compounds

앞서 얻었던 화합물 중 5 가지를 골라 농도에 따른 활성 정도를 통해 DX2 와 AIMP2 에 대한 IC50 값과 암세포와 정상 세포에 대한 GI50 값을 구해 SAP 160 번 화합물과 비교하였다. SAP-354, SAP-371, SAP-372 화합물들이 개선된 IC50 값을 갖는 것을 확인하였다. SAP-371, SAP-372 화합물은 AIMP2 대비 DX2 에 대한 선택성을 3–4 배 갖는 것을 알 수 있었으며, WI-26 cell 에 대한 GI50 값 대비 A549 cell 에 대한 GI50 값이 좋은 선택성을 보이며 개선된 것을 알 수 있었다.

III. 결론

폐암치료제로써 AIMP2-DX2 를 저해하는 저분자 화합물을 개발하기 위해 본 실험실이 구축한 라이브러리에서 SAP 160 화합물을 선도 물질로 도출하였다. 피리미딘 골격을 중심으로 A, B, C parts 로 나누어 modification 을 진행하였다. AIMP2-DX2 를 저해하며 암세포 사멸을 촉진하는 화합물을 얻고자 하였고, 각각 Luciferase assay 와 MTT assay 를 통해 활성을 확인하였다.

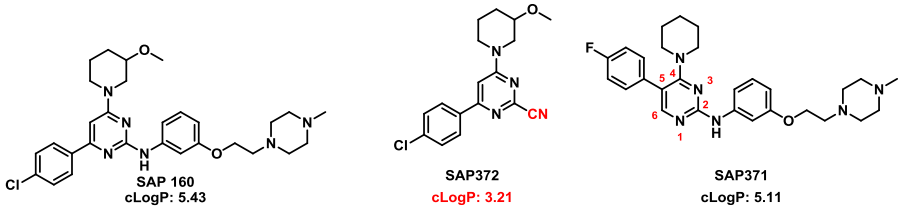


Figure 12. SAP 160 (2)으로부터 SAR을 통해 도출된 SAP 372와 SAP 371

Origin		Derivative			
Name	DX2-luciferase IC_{50} (μM)	Name	DX2-luciferase IC_{50} (μM)	AIMP2-luciferase IC_{50} (μM)	A549 MTT assay GI_{50} (μM)
BC-DXI-228	8.4	SAP371	1.1	4.1	0.9
BC-DXI-277	13.4				
BC-DXI-416	10.4	SAP372	2.7	7.7	0.98
SAP160	3.5 (GI_{50} 2.7)				

Table 7. 활성과 선택성의 개선

그 결과, Cyanide 도입된 SAP 372 화합물과 position exchange 된 SAP 371 화합물을 얻을 수 있었다. 이 두 화합물은 clogP 값이 5 와 가까워지거나 5 보다 낮아져 물성에서의 개선을 이룰 수 있었고, DX2 에 대해 개선된 IC50 값을 가지며 AIMP2 대비 선택성을 갖는다. 이 화합물들에 대해선 추후에 *in vivo* test 를 계획하고 있다.

IV. Experimental

General experimental

Unless noted otherwise, all starting materials and reagents were obtained from commercial suppliers and were used without further purification. Tetrahydrofuran was distilled from sodium benzophenone ketyl. Dichloromethane and trimethylamine were freshly distilled from calcium hydride. All solvents used for routine product isolation and chromatography were of reagent grade and glass distilled. Reaction flasks were dried at 100℃ before use, and air and moisture sensitive reactions were performed under argon. Flash column chromatography was performed using silica gel 60 (230-400 mesh, Merck) with the indicated solvents. Thin-layer chromatography was performed using 1mm silica gel plates (Merck). 1H NMR spectra were recorded on either a JEOL JNM-LA 300 (300 MHz), JEOL JNM-GCX (400 MHz), BRUKERAMX (500 MHz) or JEOL (600 MHz) spectrometers in deuteriochloroform ($CDCl_3$) or deuteriomethanol (CD_3OD). Chemical shifts are expressed in parts per million (ppm, δ) downfield from tetrametylsilane and are referenced to the deuterated solvent ($CHCl_3$). 1H NMR data are reported in the order: chemical shift, multiplicity (s, singlet; bs, broad singlet; d, doublet; dd, doublet of doublet; t, triplet; q, quartet; m,

multiplet, and/or multiple resonance), numbers of protons, and coupling constants in hertz (Hz). Mass spectra were obtained using a VG Trio-2 GC-MS instrument, and high mass spectra were obtained using a JEOL JMS-AX 505WA unit.

1. Biological Experiments

1.1 MTT assay

H460 cells (1×104 cells) were seeded in 96-well plates and treated with compounds for the indicated times in 0.5% serumcontaining medium. MTT (USB) stock solution (5 mg/ml) was 10-fold diluted and 10 μl of diluted solution was added to each well containing 200 μl of medium and incubated for 30 min. The precipitated crystal was dissolved in 100 μl of DMSO (Sigma). Absorbance was measured at 420 nm using a microplate reader (Sunrise, TECAN).

1.2 Pull-down assay for Chemical compounds and RNA complex

SynthesizedRNAfragments (2 μg) were added to the biotinylated BC-DXI01 in the RNA-binding buffer (200 mM KCl, 20 mM NaCl, 0.05% Nonidet P40, 10% glycerol, 2 mM DTT, 2 mMMgCl2 and 20 mMHepes, pH 7.8), and incubated for 1 h at 30◦C. After cross-linking by the addition of 1%formaldehyde at room temperature for 10 min, the RNA–BC-DXI01 complex was pulled down using streptavidin–Sepharose beads (1 h incubation with rotation). After washing and

incubation with DNase I (Fermentas) to remove contaminating DNA, RNA was extracted with phenol/chloroform and precipitated by ethanol. Each RNA fragment was detected via RT–PCR using a common forward primer, 5'-ATGCCGATGTACCAGGTAAAG-3', and the specific reverse primer described in the In vitro transcription section above.

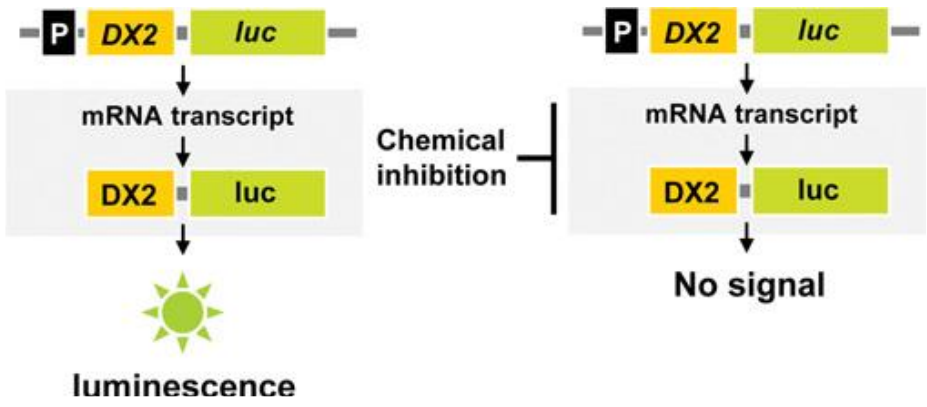


Figure S1 **Schematic diagram for the chemical screening based on luciferase assay**

AIMP2-DX2 was cloned into the pGL2 control vector, fused to the 5' end of firefly luciferase and expressed in H460 cells. The protein level of AIMP2-DX2 was monitored by luminescence (left-hand panel). When chemicals inhibit the expression of AIMP2-DX2 at the mRNA or protein level, luciferase activity is decreased and the efficacy of compounds can be calculated (right-hand panel). P, promoter.

General Procedure 1 (Suzuki coupling)

To a solution of starting material (1 equiv) and boronic acid (1.5–2 equiv) in Dimethoxyethane or THF/H_2O (1:1) solution were added Na_2CO_3 (3 equiv) and $Pd(PPh_3)_4$ (0.03 equiv). The reaction mixture was stirred at reflux condition until no starting material could be observed by TLC (ca. 4h), and diluted with EtOAc. The organic phase was washed with water and brine, dried over $MgSO_4$, and concentrated *in vacuo*. Purification of the residue via flash column chromatography on silica gel afforded titled compound.

General Procedure 2 (Nucleophilic aromatic substitution)

To a solution of starting material (1 equiv) and counter part (1.2 equiv) in EtOH was added TEA. The reaction mixture was stirred for 6 h. The reaction mixture was quenched with water, and diluted with EtOAc. The organic phase was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. Purification of the residue via column chromatography on silica gel afforded the titled compound.

General Procedure 3 (Substitution reaction)

To a solution of 2-chloropyrimidine intermediates (1 equiv) and aniline (1 equiv) in *n*-BuOH was added catalytic amount of 4*N* hydrogen chloride in 1,4-dioxane was added. The reaction mixture

was stirred for 1 h at 160℃ under microwave irradiation, and concentrated *in vacuo*. Purification of the residue via flash column chromatography on silica gel ($MeOH/CH_2Cl_2$ = 1:10 to 1:20) afforded titled *N*-aryl pyrimidine.

General Procedure 4 (Methylation)

THF was added to NaH in Ar gas to prepare suspension. Seperately, starting material was dissolved in THF andthis solution was added dropwise to above suspension at rt and the reaction mixture was sitrred for 30 min. A solution of iodomethane in THF was added dropwise, and stirred for 5 h. *Aq*.NH_4Cl solution was added to stop the reaction and water added. Organic phase was washed with brine and water, and concentrated *in vacuo*. Purification of the residue via column chromatography on silica gel afforded the titled compound.

General Procedure 5 (Boc deprotection)

To a solution of starting material (1 equiv) in CH_2Cl_2(0.1 M) was added TFA(10%). Reaction mixture was stirred for 1 h, the residue was concentrated *in vacuo*. No further purification.

General Procedure 6 (Mitsunobu reaction)

To a solution of starting material (1 equiv) and triphenylphosphine (1.3 equiv) in THF were added corresponding alcohol (1.3 equiv) and diisopropyl azodicarboxylate (DIAD) (1.3 equiv). The reaction mixture was stirred at ambient temperature until no starting material could be observed by TLC (ca. 3h). The reaction mixture was quenched with water, and diluted with EtOAc. The organic phase was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo.* Purification of the residue via column chromatography on silica gel afforded the titled compound.

General Procedure 7 (Nitro reduction)

To a solution of starting material (1 equiv) in EtOH was added $SnCl_2 \cdot 2H_2O$ (5 equiv). The reaction mixtrue was stirred for 3h at reflux condition, and concentrated *in vacuo*. The residue was diluted with EtOAc, and added *sat.* $NaHCO_3$ solution. The reaction mixture was filtered using a Celite pad, and the organic phase was washed with water and brine, dried over $MgSO_4$, and concentrated *in vacuo*. Purification of the residue via flash column chromatography on silica gel afforded titled compound.

General Procedure 8 (N-alkylation of 1-methyl piperazine)

To a solution of alkyl halide in DMF was added 1-methylpiperazine, K_2CO_3, TBAI, and stirred for overnight at 100℃. The reaction mixture was diluted with EtOAc. The organic phase was washed with water and brine, dried over $MgSO_4$, and concentrated *in vacuo*. Purification of the residue via flash column chromatography on silica gel afforded titled compound.

General Procedure 9 (Amidation)

To a solution of acid analogues in CH_2Cl_2 (0.5 M) was added DMF (*cat.*), oxalyl chloride (5 equiv). After stirring for 1hr, reaction mixture was concentrated *in vacuo*. To the reaction mixture was added THF (0.5 M), TEA (2 equiv), amine analogues (1.2 equiv) at 0 ºC. After stirring for 1, the reaction mixture was diluted with EtOAc. The organic phase was washed with water and brine, dried over $MgSO_4$, and concentrated *in vacuo*. Purification of the residue via flash column chromatography on silica gel afforded titled compound.

2,4-Dichloro-6-(4-chlorophenyl)pyrimidine (4)

2,4,6-Trichloropyrimidine **3** (0.1 ml, 0.87 mmol) afforded **4** (61 mg, 27%) by the **general procedure 1** using DME. Purification via flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:30).

2-Chloro-6-(4-chlorophenyl)-*N*,*N*-dimethylpyrimidin-4-amine (5a)

This compound was prepared by the **general procedure 2**, using 2,4-dichloro-6-(4-chlorophenyl)pyrimidine **4** (100mg, 0.39 mmol) and dimethylamine 2M in THF (0.23 ml, 0.46 mmol). Purification of the residue via flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:5) afforded **5a** (74 mg, 72%).

2-Chloro-4-(4-chlorophenyl)-6-(1*H*-pyrrol-1-yl)pyrimidine (5b)

This compound was prepared by the **general procedure 2**, using 2,4-dichloro-6-(4-chlorophenyl)pyrimidine **4** (100mg, 0.39 mmol) and pyrrole(0.03 ml, 0.46 mmol). Purification of the residue via flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:5) afforded **5b** (84 mg, 75%).

2-Chloro-4-(4-chlorophenyl)-6-(piperidin-1-yl)pyrimidine (5c)

This compound was prepared by the **general procedure 2**, using 2,4-dichloro-6-(4-chlorophenyl)pyrimidine **4** (100mg, 0.39 mmol) and piperidine (0.05ml, 0.46 mmol). Purification of the residue via flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:3) afforded **5c** (93 mg, 78%).

2-Chloro-4-(4-chlorophenyl)-6-(3-methoxypiperidin-1-yl)pyrimidine (5d)

This compound was prepared by the **general procedure 2**, using 2,4-dichloro-6-(4-chlorophenyl)pyrimidine **4** (720mg, 2.77 mmol) and 3-methoxypiperidine **12a** (421mg, 2.77 mmol). Purification of the residue via flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:3) afforded **5d** (658 mg, 70%) as pale yellow oil.

(*R*)-2-Chloro-4-(4-chlorophenyl)-6-(3-methoxypiperidin-1-yl)pyrimidine (5e)

This compound was prepared by the **general procedure 2**, using 2,4-dichloro-6-(4-chlorophenyl)pyrimidine **4** (100mg, 0.39 mmol) and (*R*)-3-methoxypiperidine **12b** (53mg, 0.46 mmol). Purification of the residue via flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:3) afforded **5e** (94 mg, 72%) as pale yellow oil.

(*S*)-2-Chloro-4-(4-chlorophenyl)-6-(3-methoxypiperidin-1-yl)pyrimidine (5f)

This compound was prepared by the **general procedure 2**, using 2,4-dichloro-6-(4-chlorophenyl)pyrimidine **4** (100mg, 0.39 mmol) and (*S*)-3-methoxypiperidine **12c** (53mg, 0.46 mmol). Purification of the residue via flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:3) afforded **5f** (90 mg, 69%) as pale yellow oil.

1-(2-Chloro-6-(4-chlorophenyl)pyrimidin-4-yl)piperidin-3-ol (5g)

This compound was prepared by the **general procedure 2**, using 2,4-dichloro-6-(4-chlorophenyl)pyrimidine **4** (300 mg, 1.16 mmol) and 3-hydroxypiperidine (117 mg, 1.16 mmol). Purification of the residue via flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:3) afforded **5g** (100 mg, 27%) as pale yellow oil.

2-Chloro-4-(4-chlorophenyl)-6-(3-methylpiperidin-1-yl)pyrimidine (5h)

This compound was prepared by the **general procedure 2**, using 2,4-dichloro-6-(4-chlorophenyl)pyrimidine **4** (100 mg, 0.39 mmol) and 3-methylpiperidine(0.05 ml, 0.46 mmol). Purification of the residue via flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:3) afforded **5h** (81 mg, 65%).

2-Chloro-4-(4-chlorophenyl)-6-(3-(trifluoromethyl)piperidin-1-yl)pyrimidine (5i)

This compound was prepared by the **general procedure 2**, using 2,4-dichloro-6-(4-chlorophenyl)pyrimidine **4** (100 mg, 0.39 mmol) and 3-(trifluoromethyl)piperidine (0.6 ml, 0.46 mmol). Purification of the residue via flash column chromatography on silica gel (EtOAc/n-Hexane = 1:3) afforded **5i** (83 mg, 67%).

2-Chloro-4-(4-chlorophenyl)-6-(3-(methoxymethyl)piperidin-1-yl)pyrimidine (5j)

This compound was prepared by the **general procedure 2**, using 2,4-dichloro-6-(4-chlorophenyl)pyrimidine **4** (100 mg, 0.39 mmol) and 3-(methoxymethyl)piperidine **12d** (60 mg, 0.46 mmol). Purification of the residue via flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:3) afforded **5j** (95 mg, 70%).

(1-(2-Chloro-6-(4-chlorophenyl)pyrimidin-4-yl)piperidin-3-yl)methanol (5k)

This compound was prepared by the **general procedure 2**, using 2,4-dichloro-6-(4-chlorophenyl)pyrimidine **4** (100 mg, 0.39 mmol) and 3-(hydroxymethyl)piperidine (0.05 ml, 0.46 mmol). Purification of the residue via flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:3) afforded **5k** (39 mg, 30%).

2-Chloro-4-(4-chlorophenyl)-6-(4-methylpiperazin-1-yl)pyrimidine (5l)

This compound was prepared by the **general procedure 2,** using 2,4-dichloro-6-(4-chlorophenyl)pyrimidine **4** (100 mg, 0.39 mmol) and 1-methylpiperazine (0.05 ml, 0.46 mmol). Purification of the residue via flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:3) afforded **5l** (56 mg, 45%).

6-(4-Chlorophenyl)-*N*4,*N*4-dimethyl-*N*2-(3-(2-(4-methylpiperazin-1-yl)ethoxy)phenyl)pyrimidine-2,4-diamine (6a)

This compound was prepared by the **general procedure 3**, using **5a** (74 mg, 0.28 mmol) and 3-(2-(4-methylpiperazin-1-yl)ethoxy)aniline **20a** (65 mg, 0.28 mmol). Purification of the residue via flash column chromatography on silica gel ($MeOH/CH_2Cl_2$ = 1:20) afforded **6a** (26 mg, 20%).

4-(4-Chlorophenyl)-*N*-(3-(2-(4-methylpiperazin-1-yl)ethoxy)phenyl)-6-(1H-pyrrol-1-yl)pyrimidin-2-amine (6b)

This compound was prepared by the **general procedure 3**, using **5b** (84 mg, 0.29 mmol) and 3-(2-(4-methylpiperazin-1-yl)ethoxy)aniline **20a** (68 mg, 0.29 mmol). Purification of the residue via flash column chromatography on silica gel ($MeOH/CH_2Cl_2$ = 1:20) afforded **6b** (23 mg, 16%).

4-(4-Chlorophenyl)-*N*-(3-(2-(4-methylpiperazin-1-yl)ethoxy)phenyl)-6-(piperidin-1-yl)pyrimidin-2-amine (6c)

This compound was prepared by the **general procedure 3**, using **5c** (93 mg, 0.30 mmol) and 3-(2-(4-methylpiperazin-1-yl)ethoxy)aniline **20a** (71 mg, 0.30 mmol). Purification of the residue via flash column chromatography on silica gel ($MeOH/CH_2Cl_2$ = 1:20) afforded 6c (26 mg, 17%) as pale yellow oil.

4-(4-Chlorophenyl)-6-(3-methoxypiperidin-1-yl)-*N*-(3-(2-(4-methylpiperazin-1-yl)ethoxy)phenyl)pyrimidin-2-amine (2)

This compound was prepared by the **general procedure 3**, using **5d** (100 mg, 0.30 mmol) and 3-(2-(4-methylpiperazin-1-yl)ethoxy)aniline **20a** (70 mg, 0.30 mmol). Purification of the residue via flash column chromatography on silica gel ($MeOH/CH_2Cl_2$ = 1:20) afforded **6d** (35 mg, 22%) as pale yellow oil: ^1H NMR (400MHz, CD_3OD): 1H NMR (400MHz, CD_3OD): δ 7.97 (d, 2H, J=8.6 Hz), 7.61 (s, 1H), 7.41 (d, 2H, J=8.5 Hz), 7.12–7.11 (m, 2H), 6.56 (s, 1H), 6.52–6.48 (m, 1H), 4.82 (s, 3H), 4.14 (s, 1H), 4.09 (t, 2H, J=5.6 Hz), 3.81–3.77 (m, 1H), 3.4–3.41 (m, 1H), 3.36 (s, 3H), 2.78 (t, 2H, J=5.5 Hz), 2.62 (brds, 4H), 2.51 (brds, 4H), 2.27 (s, 3H), 2.00–1.96 (m, 1H), 1.83–1.78 (m, 1H), 1.64–1.58 (m, 1H), 1.56–1.46 (m, 1H).

(*R*)-4-(4-Chlorophenyl)-6-(3-methoxypiperidin-1-yl)-*N*-(3-(2-(4-methylpiperazin-1-yl)ethoxy)phenyl)pyrimidin-2-amine (6e)

This compound was prepared by the **general procedure 3**, using **5e** (28 mg, 0.08 mmol) and 3-(2-(4-methylpiperazin-1-yl)ethoxy)aniline **20a** (19 mg, 0.08 mmol). Purification of the residue via flash column chromatography on silica gel ($MeOH/CH_2Cl_2$ = 1:20) afforded **6e** (3.4 mg, 8%) as pale yellow oil: ^1H NMR (400MHz, CD_3OD): δ 7.98 (d, 2H, J=8.5 Hz), 7.62 (s, 1H), 7.42 (d, 2H, J=8.6

Hz), 7.12-7.11 (m, 2H), 6.57 (s, 1H), 6.51–6.48 (m, 1H), 4.15 (s, 1H), 4.10 (t, 2H, J=5.5 Hz), 3.82–3.79 (m, 1H), 3.45–3.39 (m, 1H), 3.37 (s, 3H), 2.79 (t, 2H, J=5.5 Hz), 2.63 (brds, 4H), 2.53 (brds, 4H), 2.28 (s, 3H), 2.00 (m, 1H), 1.84–1.79 (m, 1H), 1.65–1.56 (m, 1H), 1.55–1.49 (m, 1H).

(*S*)-4-(4-Chlorophenyl)-6-(3-methoxypiperidin-1-yl)-*N*-(3-(2-(4-methylpiperazin-1-yl)ethoxy)phenyl)pyrimidin-2-amine (6f)

This compound was prepared by the **general procedure 3**, using **5f** (56 mg, 0.17 mmol) and 3-(2-(4-methylpiperazin-1-yl)ethoxy)aniline **20a** (39 mg, 0.17 mmol). Purification of the residue via flash column chromatography on silica gel ($MeOH/CH_2Cl_2$ = 1:20) afforded **6f** (3 mg, 3%) as pale yellow oil: ^1H NMR (400MHz, CD_3OD): δ 7.97 (d, 2H, J=8.6 Hz), 7.61 (s, 1H), 7.41 (d, 2H, J=8.5 Hz), 7.14–7.10 (m, 2H), 6.56 (s, 1H), 6.51–6.48 (m, 1H), 4.14 (s, 1H), 4.9 (t, 2H, J=5.5 Hz), 3.80–3.77 (m, 1H), 3.42–3.39 (m, 1H), 3.36 (s, 3H), 2.78 (t, 2H, J=5.5 Hz), 2.63 (brds, 4H), 2.53 (brds, 4H), 2.29 (s, 3H), 2.00–1.96 (m, 1H), 1.82–1.78 (m, 1H), 1.64–1.55 (m, 1H), 1.53–1.46 (m, 1H).

1-(6-(4-Chlorophenyl)-2-((3-(2-(4-methylpiperazin-1-yl)ethoxy)phenyl)amino)pyrimidin-4-yl)piperidin-3-ol (6g)

This compound was prepared by the **general procedure 3**, using **5g** (100 mg, 0.31 mmol) and 3-(2-(4-methylpiperazin-1-yl)ethoxy)aniline **20a** (73 mg, 0.31 mmol). Purification of the residue via flash column chromatography on silica gel ($MeOH/CH_2Cl_2$ = 1:20) afforded **6g** (8 mg, 5%).

4-(4-Chlorophenyl)-*N*-(3-(2-(4-methylpiperazin-1-yl)ethoxy)phenyl)-6-(3-methylpiperidin-1-yl)pyrimidin-2-amine (6h)

This compound was prepared by the **general procedure 3**, using **5h** (81 mg, 0.25 mmol) and 3-(2-(4-methylpiperazin-1-yl)ethoxy)aniline **20a** (59 mg, 0.25 mmol). Purification of the residue via flash column chromatography on silica gel ($MeOH/CH_2Cl_2$ = 1:20) afforded **6h** (5.3 mg, 4%).

4-(4-Chlorophenyl)-*N*-(3-(2-(4-methylpiperazin-1-yl)ethoxy)phenyl)-6-(3-(trifluoromethyl)piperidin-1-yl)pyrimidin-2-amine (6i)

This compound was prepared by the **general procedure 3**, using **5i** (83 mg, 0.22 mmol) and 3-(2-(4-methylpiperazin-1-yl)ethoxy)aniline **20a** (52 mg, 0.22 mmol). Purification of the residue via flash column chromatography on silica gel ($MeOH/CH_2Cl_2$ = 1:20) afforded **6i** (7 mg, 5%): ^{1}H NMR (400MHz, $CDCl_3$): δ 7.91 (d,

2H, J=8.5 Hz), 7.41 (d, 3H, J=8.4 Hz), 7.17 (t, 1H, J=8.0 Hz), 7.11 (d, 1H, J=8.1 Hz), 6.98 (s, 1H), 6.54 (dd, 1H, J=7.6, 1.4 Hz), 6.42 (s, 1H), 4.78 (d, 1H, J=12.2 Hz), 4.32 (d, 1H, J=13 Hz), 4.11 (t, 2H, J=6.1 Hz), 2.97–2.85 (m, 2H), 2.81 (t, 2H, J=5.8 Hz), 2.64 (brds, 4H), 2.52 (brds, 4H), 2.31 (s, 3H), 2.12–2.09 (m, 1H), 2.02–2.00 (m, 1H), 1.89–1.85 (m, 1H), 1.69–1.56 (m, 2H).

4-(4-Chlorophenyl)-6-(3-(methoxymethyl)piperidin-1-yl)-*N*-(3-(2-(4-methylpiperazin-1-yl)ethoxy)phenyl)pyrimidin-2-amine (6j)

This compound was prepared by the **general procedure 3**, using **5j** (95 mg, 0.27 mmol) and 3-(2-(4-methylpiperazin-1-yl)ethoxy)aniline **20a** (63 mg, 0.27 mmol). Purification of the residue via flash column chromatography on silica gel ($MeOH/CH_2Cl_2$ = 1:20) afforded **6j** (7 mg, 5%): ^1H NMR (400MHz, $CDCl_3$): δ 7.91 (d, 2H, J=8.4 Hz), 7.52 (s, 1H), 7.40 (d, 2H, J=8.4 Hz), 7.16 (t, 1H, J=8.1 Hz), 7.07 (d, 1H, J=8.0 Hz), 6.99 (s, 1H), 6.52 (d, 1H, J=8.1 Hz), 6.43 (s, 1H), 4.23 (t, 2H, J=14.2 Hz), 4.14–4.07 (m, 2H), 3.33 (s, 3H), 3.19–3.13 (m, 1H), 2.94 (dd, 1H, J=13.0, 9.6 Hz), 2.82 (t, 2H, J=5.7 Hz), 2.68 (brds, 4H), 2.57 (brds, 4H), 2.34 (s, 3H), 2.30–2.23 (m, 3H), 1.77–1.58 (m, 3H), 1.48–1.41 (m, 1H).

(1-(6-(4-Chlorophenyl)-2-((3-(2-(4-methylpiperazin-1-yl)ethoxy)phenyl)amino)pyrimidin-4-yl)piperidin-3-yl)methanol (6k)

This compound was prepared by the **general procedure 3**, using **5k** (39 mg, 0.12 mmol) and 3-(2-(4-methylpiperazin-1-yl)ethoxy)aniline **20a** (27 mg, 0.12 mmol). Purification of the residue via flash column chromatography on silica gel ($MeOH/CH_2Cl_2$ = 1:20) afforded **6k** (2 mg, 3%).

4-(4-Chlorophenyl)-6-(4-methylpiperazin-1-yl)-*N*-(3-(2-(4-methylpiperazin-1-yl)ethoxy)phenyl)pyrimidin-2-amine (6l)

This compound was prepared by the **general procedure 3**, using **5l** (56 mg, 0.17 mmol) and 3-(2-(4-methylpiperazin-1-yl)ethoxy)aniline **20a** (41 mg, 0.17 mmol). Purification of the residue via flash column chromatography on silica gel ($MeOH/CH_2Cl_2$ = 1:20) afforded **6l** (7mg, 7%).

2-Chloro-4-(4-chlorophenyl)pyrimidine (8a)

2,4-dichloropyrimidine **7a** (200 mg, 1.34 mmol) afforded **8a** (217 mg, 72 %) via **general procedure 1** using DME. Purification via flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:30)

2-Chloro-4-(4-chlorophenyl)-6-methylpyrimidine (8b)

2,4-dichloro-6-methylpyrimidine **7b** (200 mg, 1.23 mmol) afforded **8b** (217 mg, 74%) by the **general procedure 1** using DME. Purification via flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:30)

4-(4-Chlorophenyl)-*N*-(3-(2-(4-methylpiperazin-1-yl)ethoxy)phenyl)pyrimidin-2-amine (1)

This compound was prepared by the **general procedure 3**, using **8a** (217 mg, 0.97 mmol) and 3-(2-(4-methylpiperazin-1-yl)ethoxy)aniline **20a** (227 mg, 0.97 mmol). Purification of the residue via flash column chromatography on silica gel ($MeOH/CH_2Cl_2$ = 1:20) afforded **1** (106 mg, 26 %): ^1H NMR (400MHz, CD_3OD): δ 8.46 (d, 1H, J=5.2 Hz), 8.15 (d, 2H, J=6.7 Hz), 7.64 (s, 1H), 7.52 (d, 2H, J=6.8 Hz), 7.28 (d, 1H, J=5.4 Hz), 7.20–7.19 (m, 2H), 6.61–6.58 (m, 1H), 4.17 (t, 2H, J=5.5 Hz), 2.86 (t, 2H, J=5.5 Hz), 2.69 (brds, 4H), 2.61 (brds, 4H), 2.35 (s, 3H).

4-(4-Chlorophenyl)-6-methyl-*N*-(3-(2-(4-methylpiperazin-1-yl)ethoxy)phenyl)pyrimidin-2-amine (9)

This compound was prepared by the **general procedure 3** , using **8b** (217mg, 0.91 mmol) and 3-(2-(4-methylpiperazin-1-yl)ethoxy)aniline **20a** (214 mg, 0.91 mmol). Purification of the

residue via flash column chromatography on silica gel ($MeOH/CH_2Cl_2$ = 1:20) afforded **9** (68 mg, 17%).

***tert*-Butyl 3-methoxypiperidine-1-carboxylate (11a)**

This compound was prepared by the **general procedure 4**, using tert-butyl 3-hydroxypiperidine-1-carboxylate **10a** (200 mg, 1 mmol). Purification of the residue via flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:3) afforded **11a** (186 mg, 87%).

***tert*-Butyl (*R*)-3-methoxypiperidine-1-carboxylate (11b)**

This compound was prepared by the **general procedure 4**, using tert-butyl (*R*)-3-hydroxypiperidine-1-carboxylate **10b** (200 mg, 1 mmol). Purification of the residue via flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:3) afforded **11b** (178 mg, 83%).

***tert*-Butyl (*S*)-3-methoxypiperidine-1-carboxylate (11c)**

This compound was prepared by the **general procedure 4**, using tert-butyl (*S*)-3-hydroxypiperidine-1-carboxylate **10c** (200 mg, 1 mmol). Purification of the residue via flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:3) afforded **11c** (180 mg, 84%).

***tert*-Butyl 3-(methoxymethyl)piperidine-1-carboxylate (11d)**

This compound was prepared by the **general procedure 4**, using tert-butyl 3-(hydroxymethyl)piperidine-1-carboxylate **10d** (200 mg, 0.93 mmol). Purification of the residue via flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:3) afforded **11d** (183 mg, 86%).

3-Methoxypiperidine (12a)

11a (186 mg, 0.86 mmol) afforded **12a** (99 mg, 99%) as white solid via **general procedure 5**.

(*R*)-3-Methoxypiperidine (12b)

11b (178 mg, 0.83 mmol) afforded **12b** (94 mg, 99%) as white solid via **general procedure 5**.

(*S*)-3-Methoxypiperidine (12c)

11c (180 mg, 0.84 mmol) afforded **12c** (95 mg, 99%) as white solid via **general procedure 5**.

3-(Methoxymethyl)piperidine (12d)

11d (183 mg, 0.80 mmol) afforded **12d** (102 mg, 99%) as white solid via **general procedure 5**.

2,4-Dichloro-6-(2-fluorophenyl)pyrimidine (13a)

2,4,6-Trichloropyrimidine **3** (0.1 ml, 0.87 mmol) afforded **13a** (161 mg, 76%) via **general procedure 1** using 2-fluorophenylboronic acid (122 mg, 0.87 mmol). Purification via flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:30).

2,4-Dichloro-6-(3,5-difluorophenyl)pyrimidine (13b)

2,4,6-Trichloropyrimidine **3** (0.1 ml, 0.87 mmol) afforded **13b** (145 mg, 64%) via **general procedure 1** using 3, 5-difluorophenylboronic acid (137 mg, 0.87 mmol). Purification via flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:30).

4-(2,6-Dichloropyrimidin-4-yl)phenol (13c)

2,4,6-Trichloropyrimidine **3** (0.1 ml, 0.87 mmol) afforded **13c** (67 mg, 32%) via **general procedure 1** using 4-hydroxyphenylboronic acid (120 mg, 0.87 mmol). Purification via flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:30)

(*E*)-2,4-dichloro-6-(prop-1-en-1-yl)pyrimidine (13d)

2,4,6-Trichloropyrimidine **3** (0.1 ml, 0.87 mmol) afforded **13d** (99 mg, 60%) via **general procedure 1** using trnas-propenylboronic acid (74.7 mg, 0.87mmol). Purification via flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:30)

2,6-Dichloro-*N*,*N*-diethylpyrimidin-4-amine (13e)

2,4,6-Trichloropyrimidine **3** (0.1 ml, 0.87 mmol) afforded **13e** (117 mg, 61%) via **general procedure 2** using diethylamine 2M in THF (0.44 ml, 0.87 mmol). Purification via flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:3)

***N*-Butyl-2,6-dichloropyrimidin-4-amine (13f)**

2,4,6-Trichloropyrimidine **3** (0.1 ml, 0.87 mmol) afforded **13f** (107 mg, 56%) via **general procedure 2** using butylamine (0.08 ml, 0.87mmol). Purification via flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:3)

2-Chloro-4-(2-fluorophenyl)-6-(3-methoxypiperidin-1-yl)pyrimidine (14a)

This compound was prepared by the **general procedure 2**, using **12a** (62 mg, 0.41 mmol) and **13a** (100 mg, 0.41 mmol). Purification of the residue via flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:5) afforded **14a** (80 mg, 60%).

2-Chloro-4-(3,5-difluorophenyl)-6-(3-methoxypiperidin-1-yl)pyrimidine (14b)

This compound was prepared by the **general procedure 2**, using **12a** (58.1 mg, 0.38 mmol) and **13b** (100 mg, 0.38 mmol). Purification

of the residue via flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:3) afforded **14b** (80 mg, 62%).

4-(2-Chloro-6-(3-methoxypiperidin-1-yl)pyrimidin-4-yl)phenol (14c)

This compound was prepared by the **general procedure 2**, using **12a** (32 mg, 0.28 mmol) and **13c** (67 mg, 0.28 mmol). Purification of the residue via flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:3) afforded **14c** (41 mg, 46%).

(*E*)-2-Chloro-4-(3-methoxypiperidin-1-yl)-6-(prop-1-en-1-yl)pyrimidine (14d)

This compound was prepared by the **general procedure 2**, using **12a** (60 mg, 0.52 mmol) and **13d** (99 mg, 0.52 mmol). Purification of the residue via flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:3) afforded **14d** (90 mg, 64%).

2-Chloro-*N*,*N*-diethyl-6-(3-methoxypiperidin-1-yl)pyrimidin-4-amine (14e)

This compound was prepared by the **general procedure 2**, using **12a** (61 mg, 0.53 mmol) and **13e** (117 mg, 0.53 mmol). Purification of the residue via flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:3) afforded **14e** (76 mg, 48%).

***N*-Butyl-2-chloro-6-(3-methoxypiperidin-1-yl)pyrimidin-4-amine (14f)**

This compound was prepared by the **general procedure 2**, using **12a** (56 mg, 0.49 mmol) and **13f** (107mg, 0.49 mmol). Purification of the residue via flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:3) afforded **14f** (93 mg, 64%) as pale yellow oil.

4-(2-Fluorophenyl)-6-(3-methoxypiperidin-1-yl)-*N*-(3-(2-(4-methylpiperazin-1-yl)ethoxy)phenyl)pyrimidin-2-amine (15a)

This compound was prepared by the **general procedure 3**, using **14a** (30 mg, 0.09 mmol) and 3-(2-(4-methylpiperazin-1-yl)ethoxy)aniline **20a** (23 mg, 0.09 mmol). Purification of the residue via flash column chromatography on silica gel ($MeOH/CH_2Cl_2$ = 1:20) afforded **15a** (3.4 mg, 7%): ^1H NMR (400MHz, CD_3OD): δ 7.94 (td, 1H, J=7.8, 1.7 Hz), 7.59 (t, 1H, J=1Hz), 7.46–7.40 (m, 1H), 7.26 (td, 1H, J=7.6, 0.8 Hz) 7.21–7.16 (m, 1H), 7.13–7.10 (m, 2H), 6.57 (d, 1H, J=0.7 Hz), 6.54–6.49 (m, 1H), 4.10–4.06 (m, 1H), 3.80–3.75 (m, 1H), 3.51–3.43 (m, 2H), 3.38 (s, 3H), 3.37–3.32 (m, 1H), 2.81 (t, 2H, J=5.3 Hz), 2.66 (brds, 4H), 2.57 (brds, 4H), 2.32 (s, 3H), 2.03–1.99 (m, 1H), 1.86–1.79 (m, 1H), 1.68–1.48 (m, 2H).

4-(3,5-Difluorophenyl)-6-(3-methoxypiperidin-1-yl)-*N*-(3-(2-(4-methylpiperazin-1-yl)ethoxy)phenyl)pyrimidin-2-amine (15b)

This compound was prepared by the **general procedure 3**, using **14b** (40 mg, 0.12 mmol). Purification of the residue via flash column chromatography on silica gel ($MeOH/CH_2Cl_2$ = 1:20) afforded **15b** (6.6 mg, 10%): ^1H NMR (300MHz, CD_3OD): δ 7.71–7.64 (m, 3H), 7.15–7.12 (m, 2H), 7.05–6.99 (m, 1H), 6.68 (s, 1H), 6.55–6.52 (m, 1H), 4.15 (t, 3H, J=5.3 Hz), 3.82 (m, 1H), 3.54–3.47 (m, 3H), 3.40 (s, 3H), 2.84 (t, 2H, J=5.3 Hz), 2.69 (brds, 4H), 2.59 (brds, 4H), 2.33 (s, 3H), 2.00 (m, 1H), 1.83 (m, 1H), 1.66-1.56 (m, 2H).

4-(6-(3-Methoxypiperidin-1-yl)-2-((3-(2-(4-methylpiperazin-1-yl)ethoxy)phenyl)amino)pyrimidin-4-yl)phenol (15c)

This compound was prepared by the **general procedure 3**, using **14c** (20 mg, 0.06 mmol) and 3-(2-(4-methylpiperazin-1-yl)ethoxy)aniline **20a** (15 mg, 0.06 mmol). Purification of the residue via flash column chromatography on silica gel ($MeOH/CH_2Cl_2$ = 1:20) afforded **15c** (1.6 mg, 5%): ^1H NMR (500MHz, CD_3OD): δ 8.11 (d, 2H, J=8.6 Hz), 7.67 (s, 1H), 7.25 (d, 2H, J=8.6 Hz), 7.16-7.12 (m, 2H), 6.65 (s, 1H), 6.53–6.51 (m, 1H), 6.17 (s, 1H), 4.20 (m, 1H), 4.15 (t, 2H, J=5.6 Hz), 3.87–3.84 (m, 1H), 3.52–3.44 (m, 1H), 3.41 (s, 3H), 3.39–3.35 (m, 1H), 2.82 (t, 2H, J=5.5 Hz), 2.62 (brds, 4H), 2.51 (brds, 4H), 2.26 (s, 3H), 2.06-2.02 (m, 1H), 1.88–1.84 (m, 1H), 1.69–1.53 (m, 2H), 1.39–1.37 (m, 1H).

(*E*)-4-(3-Methoxypiperidin-1-yl)-*N*-(3-(2-(4-methylpiperazin-1-yl)ethoxy)phenyl)-6-(prop-1-en-1-yl)pyrimidin-2-amine (15d)

This compound was prepared by the **general procedure 3**, using **14d** (20 mg, 0.07 mmol) and 3-(2-(4-methylpiperazin-1-yl)ethoxy)aniline **20a** (18 mg, 0.07 mmol). Purification of the residue via flash column chromatography on silica gel ($MeOH/CH_2Cl_2$ = 1:20) afforded **15d** (3.5 mg, 10%) as pale yellow oil.

***N*4,*N*4-Diethyl-6-(3-methoxypiperidin-1-yl)-*N*2-(3-(2-(4-methylpiperazin-1-yl)ethoxy)phenyl)pyrimidine-2,4-diamine (15e)**

This compound was prepared by the **general procedure 3**, using **14e** (30 mg, 0.10 mmol) and 3-(2-(4-methylpiperazin-1-yl)ethoxy)aniline **20a** (24 mg, 0.10 mmol). Purification of the residue via flash column chromatography on silica gel ($MeOH/CH_2Cl_2$ = 1:20) afforded **15e** (2 mg, 5%).

***N*4-Butyl-6-(3-methoxypiperidin-1-yl)-*N*2-(3-(2-(4-methylpiperazin-1-yl)ethoxy)phenyl)pyrimidine-2,4-diamine (15f)**

This compound was prepared by the **general procedure 3**, using **14f** (30 mg, 0.10 mmol) and 3-(2-(4-methylpiperazin-1-yl)ethoxy)aniline **20a** (24 mg, 0.10 mmol). Purification of the residue via flash column chromatography on silica gel ($MeOH/CH_2Cl_2$ =

1:20) afforded **15f** (4 mg, 8%): ^{1}H NMR (500MHz, CD_3OD): δ 7.27 (s, 1H), 7.12 (t, 1H, J=8.1 Hz), 6.97 (d, 1H, J=8.0 Hz), 6.52 (dd, 1H, J=8.1, 2.0 Hz), 4.11 (t, 2H, J=5.4 Hz), 4.04 (d, 1H, J=12.2 Hz), 3.72–3.69 (m, 1H), 3.38 (s, 3H), 3.28–3.24 (m, 1H), 3.15-3.10 (m, 2H), 2.80 (t, 2H, J=5.4 Hz), 2.65 (brds, 4H), 2.3 (brds, 4H), 2.29 (s, 3H), 2.03-2.00 (m, 1H), 1.76–1.74 (m, 1H), 1.60–1.54 (m, 2H), 1.53–1.47 (m, 2H), 1.46–1.38 (m, 2H), 0.95 (t, 3H, J=7.4 Hz).

1-(2-Chloroethoxy)-3-nitrobenzene (17a)

This compound was prepared by the **general procedure 6**, using 3-nitrophenol **16a** (3 g, 21.57 mmol) and 2-chloroethanol (2.17 ml, 32.35 mmol). Purification of the residue via flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:10) afforded **17a** (3.63 g, 84%).

1-(2-Chloroethoxy)-4-nitrobenzene (17b)

This compound was prepared by the **general procedure 6**, using 4-nitrophenol **16b** (100mg, 0.72 mmol) and 2-chloroethanol (0.07 ml, 0.72 mmol). Purification of the residue via flash column chromatography on silica gel (EtOAc:*n*-Hexane = 1:10) afforded **17b** (116 mg, 80%).

1-(2-Chloroethoxy)-4-nitrobenzene (17c)

This compound was prepared by the **general procedure 6**, using 2-nitrophenol **16c** (200 mg, 1.44 mmol) and 2-chloroethanol (0.17 ml, 2.16 mmol). Purification of the residue via flash column chromatography on silica gel (EtOAc:*n*-Hexane = 1:10) afforded **17c** (183 mg, 63%).

1-(2-Chloroethoxy)-2-nitrobenzene (17d)

This compound was prepared by the **general procedure 6**, using 3-nitrophenol **16a** (200 mg, 1.44 mmol) and 3-chloropropanol (0.18 mmol, 2.16 mmol). Purification of the residue via flash column chromatography on silica gel (EtOAc:*n*-Hexane = 1:10) afforded **17d** (208 mg, 67%).

3-(2-Chloroethoxy)aniline (18a)

This compound was prepared by the **general procedure 7**, using **17a** (50 mg, 0.25 mmol). Purification of the residue via flash column chromatography on silica gel (MeOH/CH_2Cl_2 = 1:5) afforded **18a** (15 mg, 35%).

3-(3-Chloropropoxy)aniline (18d)

This compound was prepared by the **general procedure 7**, using **17d** (50 mg, 0.23 mmol). Purification of the residue via flash column

chromatography on silica gel (MeOH/CH_2Cl_2 = 1:5) afforded **18d** (17 mg, 39%).

1-Methyl-4-(2-(3-nitrophenoxy)ethyl)piperazine (19a)

This compound was prepared by the **general procedure 8**, using **17a** (3.63 g, 18.02 mmol) and 1-methylpiperazine (6 ml, 54.05 mmol). Purification of the residue via flash column chromatography on silica gel (MeOH/CH_2Cl_2 = 1:10) afforded **19a** (2.18 g, 46%).

1-Methyl-4-(2-(4-nitrophenoxy)ethyl)piperazine (19b)

This compound was prepared by the **general procedure 8**, using **17b** (116mg, 0.58 mmol) and 1-methylpiperazine (0.19 ml, 1.73 mmol). Purification of the residue via flash column chromatography on silica gel (MeOH/CH_2Cl_2 = 1:10) afforded **19b** (87 mg, 57%).

1-Methyl-4-(2-(2-nitrophenoxy)ethyl)piperazine (19c)

This compound was prepared by the **general procedure 8**, using **17c** (183 mg, 0.91 mmol), 1-methylpiperazine (0.30 ml, 2.72 mmol). Purification of the residue via flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:10) afforded **19c** (120 mg, 50%).

1-Methyl-4-(2-(3-nitrophenoxy)propyl)piperazine (19d)

This compound was prepared by the **general procedure 8**, using **17d** (208 mg, 0.96 mmol) and 1-methylpiperazine (0.16 ml, 1.44 mmol). Purification of the residue via flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:10) afforded **19d** (132 mg, 49%).

3-(2-(4-Methylpiperazin-1-yl)ethoxy)aniline (20a)

This compound was prepared by the **general procedure 7**, using **19a** (1.10 g, 4.16 mmol). Purification of the residue via flash column chromatography on silica gel (MeOH/CH_2Cl_2 = 1:5) afforded **20a** (880 mg, 90%).

4-(2-(4-Methylpiperazin-1-yl)ethoxy)aniline (20b)

This compound was prepared by the **general procedure 7**, using **19b** (200 mg, 0.75 mmol). Purification of the residue via flash column chromatography on silica gel (MeOH/CH_2Cl_2 = 1:5) afforded **20b** (54 mg, 30%) as orange oil.

2-(2-(4-Methylpiperazin-1-yl)ethoxy)aniline (20c)

This compound was prepared by the **general procedure 7**, using **19c** (200 mg, 0.75 mmol). Purification of the residue via flash column chromatography on silica gel (MeOH/CH_2Cl_2 = 1:5) afforded **20c** (46 mg, 26%).

3-(2-(4-Methylpiperazin-1-yl)propoxy)aniline (20d)

This compound was prepared by the **general procedure 7**, using **19d** (132 mg, 0.47 mmol). Purification of the residue via flash column chromatography on silica gel ($MeOH/CH_2Cl_2$ = 1:5) afforded **20d** (40 mg, 34%).

1-Methyl-4-(3-nitrophenyl)piperazine (22)

To a solution of 1-fluoro-3-nitrobenzene **21** (0.5 ml, 4.70 mmol) in DMSO (15 ml) was added K_2CO_3 (3.24 g, 23.48 mmol) and 1-methylpiperazine (2.6 ml, 23.48 mmol). Purification of the residue via flash column chromatography on silica gel ($MeOH/CH_2Cl_2$ = 1:20) afforded **22** (181 mg, 18%).

3-(4-Methylpiperazin-1-yl)aniline (23)

To a soultion of **22** (181 mg, 0.82 mmol) in MeOH was added Pd/C. The reaction mixture was stirred for 1 h under H_2 pressure, filtered through celite , washed with CH_2Cl_2 and the filterate was concentrated. Purification of the residue via flash column chromatography on silica gel ($MeOH/CH_2Cl_2$ = 1:10) afforded **23** (142 mg, 91%).

1-(4-Methylphenethoxy)-3-nitrobenzene (24a)

This compound was prepared by the **general procedure 6**, using 3-nitrophenol **16a** (100 mg, 0.72 mmol) and 2-(4-methylphenyl)-ethanol (0.15 ml, 1.08 mmol). Purification of the residue via flash column chromatography on silica gel (EtOAc:*n*-Hexane = 1:10) afforded **24a** (124 mg, 67%).

1-(2-Cyclohexylethoxy)-3-nitrobenzene (24b)

This compound was prepared by the **general procedure 6**, using 3-nitrophenol **16a** (100 mg, 0.72 mmol) and 2-cyclohexylethanol (0.15 ml, 1.08 mmol). Purification of the residue via flash column chromatography on silica gel (EtOAc:*n*-Hexane = 1:10) afforded **24b** (106 mg, 59%).

3-(4-Methylphenethoxy)aniline (25a)

This compound was prepared by the **general procedure 7**, using **24a**. (124 mg, 0.48 mmol) Purification of the residue via flash column chromatography on silica gel (MeOH/CH_2Cl_2 = 1:5) afforded **25a** (44 mg, 40%).

3-(2-Cyclohexylethoxy)aniline (25b)

This compound was prepared by the **general procedure 7**, using **24b** (106 mg, 0.42 mmol). Purification of the residue via flash

column chromatography on silica gel ($MeOH/CH_2Cl_2$ = 1:5) afforded **25b** (47 mg, 50%).

(4-Methylpiperazin-1-yl)(4-nitrophenyl)methanone (27a)

This compound was prepared by the **general procedure 9**, using 4-nitrobenzoic acid **26a** (200 mg, 1.20 mmol) and 1-methylpiperazine (0.16 ml, 1.44 mmol). Purification of the residue via flash column chromatography on silica gel ($MeOH/CH_2Cl_2$ = 1:20) afforded **27a** (242 mg, 81%) as yellow solid.

(4-Methylpiperazin-1-yl)(3-nitrophenyl)methanone (27b)

This compound was prepared by the procedure for **27a**, using 3-nitrobenzoic acid **26b** (200 mg, 1.20 mmol) and 1-methylpiperazine (0.16 ml, 1.44 mmol). Purification of the residue via flash column chromatography on silica gel ($MeOH/CH_2Cl_2$= 1:20) afforded **27b** (234 mg, 79%).

(4-Aminophenyl)(4-methylpiperazin-1-yl)methanone (28a)

To a solution of (4-methylpiperazin-1-yl)(4-nitrophenyl)methanone **27a** (242 mg, 0.97 mmol) in MeOH was added Pd/C (24.2 mg). Stirred for 1 hr under H_2 pressure. The reaction mixture was filtered through celite, washed with CH_2Cl_2 and the filterate was concentrated in vacuo. Purification of the residue via

flash column chromatography on silica gel ($MeOH/CH_2Cl_2$ = 1:9 to 1:3) afforded **28a** (198 mg, 93%) as white solid.

(3-Aminophenyl)(4-methylpiperazin-1-yl)methanone (28b)

This compound was prepared by same procedure for **28a**, using (4-methylpiperazin-1-yl)(3-nitrophenyl)methanone **27b** (234 mg, 0.94 mmol) instead of **27a**. Purification of the residue via flash column chromatography on silica gel ($MeOH/CH_2Cl_2$ = 1:9 to 1:3) afforded **28b** (195 mg, 92%)

4-(4-Chlorophenyl)-6-(3-methoxypiperidin-1-yl)pyrimidin-2-amine (29)

To a solution of 2-chloro-4-(4-chlorophenyl)-6-(3-methoxypiperidin-1-yl)pyrimidine **5d** (100mg, 0.30 mmol) in 1,4-dioxane 0.5ml was added NH_4OH solution 1ml. The reaction mixture was stirred for 1 h 30 min at 140℃ under microwave irradiation, and concentrated *in vacuo*. Purification of the residue via flash column chromatography on silica gel ($MeOH/CH_2Cl_2$ = 1:9 to 1:3) afforded **29** (38 mg, 40%).

4-(4-Chlorophenyl)-6-(3-methoxypiperidin-1-yl)-*N*-(4-(2-(4-methylpiperazin-1-yl)ethoxy)phenyl)pyrimidin-2-amine (30a)

This compound was prepared by the **general procedure 3**, using **5d** (20 mg, 0.06 mmol) and **20b** (14 mg, 0.06 mmol). Purification of the residue via flash column chromatography on silica gel ($MeOH/CH_2Cl_2$ = 1:20) afforded **30a** (2mg, 7%): ^1H NMR (400MHz, CD_3OD): δ 7.87 (d, 2H, J=8.5 Hz), 7.50 (d, 2H, J=8.9 Hz), 7.39 (d, 2H, J=8.5 Hz), 6.85 (d, 3H, J= 8.8 Hz), 6.37 (s, 1H), 4.21 (d, 1H, J=8.3 Hz), 4.08 (t, 2H, J=5.5 Hz), 3.88 (d, 1H, J=13.2 Hz), 3.39 (s, 3H), 3.32–3.22 (m, 3H), 2.80 (t, 2H, J=5.8 Hz), 2.64 (brds, 4H), 2.51 (brds, 4H), 2.31 (s, 3H), 1.86–1.83 (m, 1H), 1.62–1.49 (m, 3H).

4-(4-Chlorophenyl)-6-(3-methoxypiperidin-1-yl)-*N*-(2-(2-(4-methylpiperazin-1-yl)ethoxy)phenyl)pyrimidin-2-amine (30b)

This compound was prepared by the **general procedure 3**, using **5d** (20 mg, 0.06 mmol) and **20c** (14mg, 0.06 mmol). Purification of the residue via flash column chromatography on silica gel ($MeOH/CH_2Cl_2$ = 1:20) afforded **30b** (2 mg, 7%): ^1H NMR (400MHz, $CDCl_3$): δ 8.52 (d, 1H, J=7.9 Hz), 7.91 (d, 2H, J=8.6 Hz), 7.74 (s, 1H), 7.40 (d, 2H, J=6.7 Hz), 6.98–6.93 (m, 1H), 6.89 (d, 2H, J=4.0 Hz), 6.40 (s, 1H), 4.27 (d, 1H, J=11.5 Hz), 4.18 (t, 2H, J=5.8 Hz), 3.92 (d, 1H, J=13.2 Hz), 3.41 (s, 3H), 3.34–3.19 (m, 3H), 2.84 (t, 2H, J=5.8 Hz), 2.64 (brds, 4H), 2.51 (brds, 4H), 2.24 (s, 3H), 2.08–2.04 (m, 1H), 1.87–1.84 (m, 1H), 1.69–1.50 (m, 2H).

***N*-(3-(2-Chloroethoxy)phenyl)-4-(4-chlorophenyl)-6-(3-methoxypiperidin-1-yl)pyrimidin-2-amine (30c)**

This compound was prepared by the **general procedure 3**, using **5d** (20 mg, 0.06 mmol) and **18a** (10 mg, 0.06 mmol). Purification of the residue via flash column chromatography on silica gel ($MeOH/CH_2Cl_2$ = 1:20) afforded **30c** (3 mg ,12%): ^1H NMR (400MHz, $CDCl_3$): δ 7.90 (d, 2H, J=4.3 Hz), 7.61 (t, 1H, J=2.1 Hz), 7.41 (d, 2H, J=4.3 Hz), 7.18 (t, 1H, J=8.1 Hz), 7.03 (dd, 1H, J=8.0, 1.1 Hz), 6.96 (s, 1H), 6.54 (dd, 1H, J=8.1, 1.9 Hz), 6.42 (s, 1H), 4.25 (t, 2H, J=6.0 Hz), 4.22–4.20 (m, 1H), 3.90 (d, 1H, J=12.4 Hz), 3.80 (t, 2H, J=6.0 Hz), 3.41 (s, 3H), 3.36–3.30 (m, 3H), 2.08–2.02 (m, 1H), 1.88-1.85 (m, 1H), 1.63–1.51 (m, 3H)

4-(4-Chlorophenyl)-*N*-(3-(3-chloropropoxy)phenyl)-6-(3-methoxypiperidin-1-yl)pyrimidin-2-amine (30d)

This compound was prepared by the **general procedure 3**, using **5d** (20 mg, 0.06 mmol) and **18d** (11 mg, 0.06 mmol). Purification of the residue via flash column chromatography on silica gel ($MeOH/CH_2Cl_2$ = 1:20) afforded **30d** (4 mg, 14%): ^1H NMR (400MHz, $CDCl_3$): δ 7.90 (d, 2H, J=8.5 Hz), 7.56 (t, 1H, J=2.0 Hz), 7.40 (d, 2H, J=8.6 Hz), 7.17 (t, 1H, J=8.1 Hz), 7.03 (d, 1H, J=7.9 Hz), 6.96 (s, 1H), 6.53 (dd, 1H, J=8.0, 1.8 Hz), 6.42 (s, 1H), 4.21 (d, 1H, J=9.1 Hz), 4.13 (t, 2H, J=5.8 Hz), 3.91 (d, 1H, J=.8 Hz), 3.74 (t, 2H,

J=6.3 Hz), 3.63–3.56 (m, 1H), 3.40 (s, 3H), 3.35–3.28 (m, 3H), 2.22 (qui, 2H, J=6.1 Hz), 2.042.01 (m, 1H), 1.88–1.85 (m, 1H), 1.58–1.52 (m, 1H).

4-(4-Chlorophenyl)-6-(3-methoxypiperidin-1-yl)-*N*-(3-(3-(4-methylpiperazin-1-yl)propoxy)phenyl)pyrimidin-2-amine (30e)

This compound was prepared by the **general procedure 3**, using **5d** (20 mg, 0.06 mmol) and **20d** (15 mg, 0.06 mmol). Purification of the residue via flash column chromatography on silica gel ($MeOH/CH_2Cl_2$ = 1:20) afforded **30e** (3mg, 9%): ^1H NMR (400MHz, $CDCl_3$): δ 7.90 (d, 2H, J=8.4 Hz), 7.49 (s, 1H), 7.39 (d, 2H, J=8.4 Hz), 7.15 (t, 1H, J=8.1 Hz), 7.04 (d, 1H, J=8.0 Hz), 6.94 (s, 1H), 6.52 (dd, 1H, J=6.4, 1.6 Hz), 6.41 (s, 1H), 4.22 (d, 1H, J=10.0 Hz), 4.02 (t, 2H, J=6.2 Hz), 3.90 (d, 1H, J=13.2 Hz), 3.40 (s, 3H), 3.33–3.26 (m, 3H), 2.54 (t, 10H, J=7.3 Hz), 2.31 (s, 3H), 2.00-1.93 (m, 2H), 1.87–1.84 (m, 1H), 1.61–1.52 (m, 2H).

4-(4-Chlorophenyl)-6-(3-methoxypiperidin-1-yl)-*N*-(3-(4-methylpiperazin-1-yl)phenyl)pyrimidin-2-amine (30f)

This compound was prepared by the **general procedure 3**, using **5d** (20 mg, 0.06 mmol) and **23** (11 mg, 0.06 mmol). Purification of the residue via flash column chromatography on silica gel ($MeOH/CH_2Cl_2$ = 1:20) afforded **30f** (3 mg, 10%).

4-(4-Chlorophenyl)-6-(3-methoxypiperidin-1-yl)-*N*-(3-(4-methylphenethoxy)phenyl)pyrimidin-2-amine (30g)

This compound was prepared by the **general procedure 3**, using **5d** (20 mg, 0.06 mmol) and **25a** (13 mg, 0.06 mmol). Purification of the residue via flash column chromatography on silica gel ($MeOH/CH_2Cl_2$ = 1:20) afforded **30g** (3 mg, 10%): ^1H NMR (300MHz, $CDCl_3$): δ 7.81 (d, 2H, J=8.6 Hz), 7.59 (t, 1H, J=2.1 Hz), 7.23 (d, 2H, J=6.8 Hz), 7.09 (t, 2H, J=7.5 Hz), 7.07 (t, 2H, J=7.9 Hz), 6.91 (s, 1H), 6.88 (dd, 1H, J=8.0, 1.1 Hz), 6.47 (dd, 1H, J=8.2, 2.4 Hz), 6.35 (s, 1H), 4.10 (t, 2H, J=7.3 Hz), 3.82 (d, 1H, J=13.0 Hz), 3.33 (s, 3H), 3.30–3.18 (m, 3H), 3.00 (t, 2H, J=7.3 Hz), 2.27 (s, 3H), 1.97–1.89 (m, 1H), 1.58–1.35 (m, 3H).

4-(4-Chlorophenyl)-*N*-(3-(2-cyclohexylethoxy)phenyl)-6-(3-methoxypiperidin-1-yl)pyrimidin-2-amine (30h)

This compound was prepared by the **general procedure 3**, using **5d** (20 mg, 0.06 mmol) and **25b** (13 mg, 0.06 mmol). Purification of the residue via flash column chromatography on silica gel ($MeOH/CH_2Cl_2$ = 1:20) afforded **30h** (5 mg, 17%): ^1H NMR (600MHz, $CDCl_3$): δ 7.89 (d, 2H, J=8.3 Hz), 7.51 (t, 1H), 7.39 (d, 2H, J=8.7 Hz), 7.15 (t, 1H, J=8.0 Hz), 6.41 (s, 1H), 4.21 (d, 1H, J=7.8 Hz), 3.99 (t, 2H, J=6.7 Hz), 3.92 (t, 1H, J=6.7 Hz), 3.89 (m, 1H), 3.41 (s, 3H), 3.32–3.28 (m, 3H), 2.06–2.03 (m, 1H), 1.86 (m, 1H), 1.76–1.74

(m, 2H), 1.70–1.61 (m, 4H), 1.55–1.49 (m, 2H), 1.27–1.21 (m, 4H), 0.99–0.93 (m, 2H).

4-(4-Chlorophenyl)-6-(3-methoxypiperidin-1-yl)-*N*-(3-(4-methylpiperazin-1-yl)propyl)pyrimidin-2-amine (30i)

This compound was prepared by the **general procedure 3**, using **5d** (20 mg, 0.06 mmol) and 3-(4-methylpiperazin-1-yl)propan-1-amine (9 mg, 0.06 mmol). Purification of the residue via flash column chromatography on silica gel ($MeOH/CH_2Cl_2$ = 1:20) afforded **30i** (2 mg, 8%): ^1H NMR (400MHz, $CDCl_3$): δ 7.89 (d, 2H, J=8.6 Hz), 7.36 (d, 2H, J=8.5 Hz), 6.26 (s, 1H), 4.19 (d, 1H, J=11.0 Hz), 3.87 (m, 5H), 3.40 (s, 3H), 3.31–3.27 (m, 1H), 3.23–3.17 (m, 2H), 2.46(t, 4H, J=4.9 Hz), 2.32 (s, 3H), 2.04–2.00 (m, 1H), 1.83–1.80 (m, 1H), 1.54–1.47 (m, 3H), 1.26–1.17 (m, 2H), 0.86–0.84 (m, 1H).

(4-((4-(4-Chlorophenyl)-6-(3-methoxypiperidin-1-yl)pyrimidin-2-yl)amino)phenyl)(4-methylpiperazin-1-yl)methanone (30j)

This compound was prepared by the **general procedure 3**, using **5d** (39 mg, 0.11 mmol) and **28a** (28 mg, 0.13 mmol). Purification of the residue via flash column chromatography on silica gel ($MeOH/CH_2Cl_2$ = 1:20) afforded **30j** (10 mg, 17%): ^1H NMR (500MHz, CD_3OD): δ 8.04 (d, 2H, J=8.6 Hz), 7.85 (d, 2H, J=8.6 Hz),

7.46 (d, 2H, J=8.6 Hz), 7.38 (d, 2H, J=8.7 Hz), 6.69 (s, 1H), 4.17 (d, 1H, J=12.4 Hz), 3.85-3.83 (m, 1H), 3.66 (s, 4H), 3.54–3.47 (m, 2H), 3.40 (s, 3H), 2.61 (t, 1H, J=4.7 Hz), 2.48 (s, 4H), 2.33 (s, 3H), 2.05–2.02 (m, 1H), 2.00 (s, 3H), 1.87–1.83 (m, 1H), 1.67–1.62 (m, 1H), 1.59–1.52 (m, 1H).

(3-((4-(4-Chlorophenyl)-6-(3-methoxypiperidin-1-yl)pyrimidin-2-yl)amino)phenyl)(4-methylpiperazin-1-yl)methanone (30k)

This compound was prepared by the **general procedure 3**, using **5d** (20 mg, 0.06 mmol) and **28b** (14 mg, 0.07 mmol). Purification of the residue via flash column chromatography on silica gel ($MeOH/CH_2Cl_2$ = 1:20) afforded **30k** (6 mg, 19%): ^1H NMR (500MHz, CD_3OD): δ 8.04 (s, 2H), 8.02 (s, 2H), 7.70 (dd, J=8.2, 2.0 Hz), 7.46 (d, 2H), 7.35 (t, 1H, J=7.9 Hz), 6.96 (d, 1H, J=7.5 Hz), 6.68 (s, 1H), 4.12 (d, 1H, J=14.5 Hz), 3.84–3.78 (m, 3H), 3.57–3.51 (m, 4H), 3.39 (s, 3H), 3.38–3.34 (m, 1H), 2.52 (s, 2H), 2.37 (s, 2H), 2.29 (s, 3H), 2.03–2.02 (m, 1H), 1.87-1.83 (m, 1H), 1.68–1.66 (m, 1H), 1.58-1.53 (m, 1H).

4-(4-Chlorophenyl)-6-(3-methoxypiperidin-1-yl)pyrimidine-2-carbonitrile (31)

To a solution of 2-chloro-4-(4-chlorophenyl)-6-(3-methoxypiperidin-1-yl)pyrimidine **5d** (286 mg, 0.85 mmol) in DMSO (5.7 ml) was added NaCN (62 mg, 1.28 mmol), DABCO (143 mg, 1.28 mmol). After stirring until no starting material could be observed by TLC (ca. 4h), and diluted with EtOAc. The organic phase was washed with water and brine, dried over MgSO4, and concentrated *in vacuo*. Purification of the residue via flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:5) afforded **31** (192mg, 69 %): ^{1}H NMR (400MHz, $CDCl_3$): δ 7.89 (d, 2H, J=8.6 Hz), 7.42 (d, 2H, J=8.6 Hz), 6.91 (s, 1H), 3.85–3.61 (m, 4H), 3.38 (s, 3H), 3.36–3.34 (m, 1H), 2.00-1.83 (m, 2H), 1.78–1.70 (m, 1H), 1.58–1.50 (m, 1H).

4-(4-Chlorophenyl)-6-(3-methoxypiperidin-1-yl)pyrimidine-2-carboxylic acid (32)

To a solution of 4-(4-chlorophenyl)-6-(3-methoxypiperidin-1-yl)pyrimidine-2-carbonitrile **31** (192 mg, 0.58 mmol) in 50% *aq*. EtOH (8ml) was added KOH (327mg, 5.83 mmol). The reaction mixture was stirred at reflux condition, and concentrated in vacuo. The residue was diluted with CH_2Cl_2 and 2*N* HCl solution, and washed with water and brine, dried over MgSO4, and concentrated in vacuo. Purification of the residue via column chromatography on silica gel (MeOH/CH_2Cl_2 = 1:10) afforded **32** (121mg, 60%): ^{1}H NMR (300MHz, CD_3OD): δ 7.93 (m, 2H), 7.50 (d, 2H, J=4.2 Hz),

7.16 (s, 1H), 3.98 (m, 2H), 3.76 (m, 2H), 3.45 (m, 1H), 3.39 (s, 3H), 3.34 (s, 1H), 2.00–1.80 (m, 4H), 1.61 (m, 1H).

4-(4-Chlorophenyl)-6-(3-methoxypiperidin-1-yl)-*N*-(3-(4-methylpiperazin-1-yl)propyl)pyrimidine-2-carboxamide (33a)

This compound was prepared by the **general procedure 9**, using 4-(4-chlorophenyl)-6-(3-methoxypiperidin-1-yl)pyrimidine-2-carbonitrile **32** (20 mg, 0.06 mmol) and 3-(4-methylpiperazin-1-yl)propan-1-amine (9.0 mg, 0.06 mmol). Purification of the residue via flash column chromatography on silica gel ($MeOH/CH_2Cl_2$ = 1:10) afforded **33a** (8.7 mg, 31%).

4-(4-Chlorophenyl)-6-(3-methoxypiperidin-1-yl)-*N*-(3-(2-(4-methylpiperazin-1-yl)ethoxy)phenyl)pyrimidine-2-carboxamide (33b)

This compound was prepared by the **general procedure 9**, using 4-(4-chlorophenyl)-6-(3-methoxypiperidin-1-yl)pyrimidine-2-carbonitrile **32** (30mg, 0.09 mmol) and **20a** (20.3 mg, 0.09 mmol). Purification of the residue via flash column chromatography on silica gel ($MeOH/CH_2Cl_2$ = 1:10) afforded **33b** (17 mg, 34 %).

4-(4-Chlorophenyl)-6-(3-methoxypiperidin-1-yl)-*N*-(4-(2-(4-methylpiperazin-1-yl)ethoxy)phenyl)pyrimidine-2-carboxamide (33c)

This compound was prepared by the **general procedure 9**, using 4-(4-chlorophenyl)-6-(3-methoxypiperidin-1-yl)pyrimidine-2-carbonitrile **32** (20 mg, 0.06 mmol) and **20b** (14 mg, 0.06 mmol). Purification of the residue via flash column chromatography on silica gel ($MeOH/CH_2Cl_2$ = 1:10) afforded **33c** (14 mg, 41%).

5-Bromo-2-chloro-4-(piperidin-1-yl)pyrimidine (35)

This compound was prepared by the **general procedure 2**, using piperidine and 5-bromo-2,4-dichloropyrimidine **34** (0.56 ml, 4.39 mmol) and piperidine (0.43 ml, 4.39 mmol). Purification of the residue via flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:3) afforded **35** (1.01 g, 83%).

2-Chloro-5-(4-fluorophenyl)-4-(piperidin-1-yl)pyrimidine (36a)

5-bromo-2-chloro-4-(piperidin-1-yl)pyrimidine **35** (250 mg, 0.90 mmol) afforded **36a** (229 mg, 87%) via **general procedure 1** using 4-fluorophenylboronic acid (126 mg, 0.90 mmol), DME. Purification via flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:10).

2-Chloro-5-(4-chlorophenyl)-4-(piperidin-1-yl)pyrimidine (36b)

5-bromo-2-chloro-4-(piperidin-1-yl)pyrimidine **35** (240 mg, 0.90 mmol) afforded **36b** (189 mg, 71%) via **general procedure 1** using 4-chlorophenylboronic acid (136 mg, 0.90 mmol), DME. Purification via flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:10).

2-Chloro-5-(4-methoxyphenyl)-4-(piperidin-1-yl)pyrimidine (36c)

5-bromo-2-chloro-4-(piperidin-1-yl)pyrimidine **35** (500 mg, 1.81 mmol) afforded **36b** (362 mg, 66%) via **general procedure 1** using 4-methoxyphenylboronic acid (275 mg, 1.81 mmol), DME. Purification via flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:10).

5-(4-Fluorophenyl)-*N*-(3-(2-(4-methylpiperazin-1-yl)ethoxy)phenyl)-4-(piperidin-1-yl)pyrimidin-2-amine (37a)

This compound was prepared by the **general procedure 3**, using **36a** (20 mg, 0.07 mmol) and **20a** (24 mg, 0.10 mmol). Purification of the residue via flash column chromatography on silica gel ($MeOH/CH_2Cl_2$ = 1:20) afforded **37a** (14 mg, 41%): ^1H NMR (500MHz, CD_3OD): δ 7.82 (s, 1H), 7.52 (t, 1H, J=2.0 Hz), 7.43–7.40

(m, 2H), 7.17-7.12 (m, 4H), 6.56–6.54 (m, 1H), 4.14 (t, 2H, J=5.4 Hz), 2.83 (t, 2H, J=5.4 Hz), 2.65 (brds, 4H), 2.54 (brds, 4H), 2.29 (s, 3H), 1.60–1.58 (m, 4H), 1.53–1.48 (m, 6H).

5-(4-Chlorophenyl)-*N*-(3-(2-(4-methylpiperazin-1-yl)ethoxy)phenyl)-4-(piperidin-1-yl)pyrimidin-2-amine (37b)

This compound was prepared by the **general procedure 3**, using **36b** (20 mg, 0.06 mmol) and **20a** (23 mg, 0.10 mmol). Purification of the residue via flash column chromatography on silica gel (MeOH/CH2Cl2 = 1:20) afforded **37b** (14 mg, 43%): ^{1}H NMR (300MHz, CD_3OD): δ 7.79 (s, 1H), 7.48–7.47 (m, 1H), 7.36 (m, 4H), 7.12–7.10 (m, 2H), 6.53–6.50 (m, 1H), 4.09 (t, 2H, J=5.4 Hz), 2.78 (t, 2H, J=5.5 Hz), 2.63 (brds, 4H), 2.51 (brds, 4H), 2.26 (s, 3H), 1.56–1.55 (m, 4H), 1.48 (brds, 6H).

4-(2-Chloro-4-(piperidin-1-yl)pyrimidin-5-yl)phenol (38)

To a solution of 2-chloro-5-(4-methoxyphenyl)-4-(piperidin-1-yl)pyrimidine **36c** (362mg, 1.19 mmol) in CH_2Cl_2 (24 ml) was added 1M BBr_3 in CH_2Cl_2 solution (2.62 ml) dropwise. The reaction mixture was stirred for 2 h, quenched by H_2O at 0℃, diluted with CH_2Cl_2, washed with H_2O and brine, and concentrated *in vacuo*. Purification of the residue via flash column chromatography on silica gel (EtOAc/*n*-Hexane = 1:3) afforded **38** (235 mg, 68%)

2-Chloro-5-(4-(4-fluorophenethoxy)phenyl)-4-(piperidin-1-yl)pyrimidine (39a)

This compound was prepared by the **general procedure 6**, using 4-(2-chloro-4-(piperidin-1-yl)pyrimidin-5-yl)phenol **38** (117 mg, 0.40 mmol) and 2-(4-fluorophenyl)ethanol (0.08 ml, 0.61 mmol). Purification of the residue via flash column chromatography on silica gel (EtOAc:*n*-Hexane = 1:3) afforded **39a** (154 mg, 93%).

2-Chloro-5-(4-(4-chlorophenethoxy)phenyl)-4-(piperidin-1-yl)pyrimidine (39b)

This compound was prepared by the **general procedure 6**, using 4-(2-chloro-4-(piperidin-1-yl)pyrimidin-5-yl)phenol **38** (117 mg, 0.40 mmol) and 2-(4-chlorophenyl)ethanol (0.08 ml, 0.61 mmol). Purification of the residue via flash column chromatography on silica gel (EtOAc:*n*-Hexane = 1:3) afforded **39b** (171 mg, 99%).

5-(4-(4-Fluorophenethoxy)phenyl)-*N*-(3-(2-(4-methylpiperazin-1-yl)ethoxy)phenyl)-4-(piperidin-1-yl)pyrimidin-2-amine (40a)

This compound was prepared by the **general procedure 3**, using **39a** (20 mg, 0.05 mmol) and **20a** (11 mg, 0.05 mmol). Purification of the residue via flash column chromatography on silica gel ($MeOH/CH_2Cl_2$ = 1:20) afforded **40a** (10 mg, 35%): ^1H NMR (300MHz, CD_3OD): δ 7.76 (s, 1H), 7.52 (d, 1H, J=2.0 Hz), 7.33–7.25

(m, 4H), 7.15–7.12 (m, 2H), 7.01 (t, 2H, J=8.8 Hz), 6.94 (d, 2H, J=8.6 Hz), 6.53 (dt, 1H, J=6.8, 2.6 Hz), 4.17 (t, 2H, J=6.7 Hz), 4.13 (t, 2H, J=5.5 Hz), 3.05 (t, J=6.6 Hz), 2.81 (t, 2H, J=5.4 Hz), 2.67 (brds, 4H), 2.53 (brds, 4H), 2.28 (s, 3H), 1.58–1.56 (m, 4H), 1.49–1.48 (m, 6H).

5-(4-(4-Chlorophenethoxy)phenyl)-*N*-(3-(2-(4-methylpiperazin-1-yl)ethoxy)phenyl)-4-(piperidin-1-yl)pyrimidin-2-amine (40b)

This compound was prepared by the **general procedure 3**, using **39b** (20 mg, 0.05 mmol) and **20a** (11 mg, 0.05 mmol). Purification of the residue via flash column chromatography on silica gel ($MeOH/CH_2Cl_2$ = 1:20) afforded **40b** (9 mg, 32%): ^1H NMR (300MHz, CD_3OD): δ 7.77 (s, 1H), 7.52–7.51 (m, 1H), 7.29–7.26 (m, 4H), 7.18–7.10 (m, 2H), 6.95 (d, 2H, J8.4 Hz), 6.54 (dt, 1H, J=7.2, 2.4 Hz), 4.20 (t, 2H, J=6.4 Hz), 4.13 (t, 2H, J=5.2 Hz), 3.06 (t, 2H, J=6.4 Hz), 2.83 (t, 2H, J=5.4 Hz), 2.68 (brds, 4H), 2.57 (brds, 4H), 2.32 (s, 3H), 1.57 (brds, 4H), 1.49 (brds, 6H).

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Abstract

AIMP2(ARS Interacting Multi-functional Protein2), also known as P38, is one of the auxiliary proteins of aminoacyl tRNA synthetase and is involved in protein synthesis as a complex called MSC(Multi-tRNA Synthetase Complex). In addition, AIMP2 is known to enhance tumor suppression signal by TGF-beta and to inhibit ubiquitination of p53, which plays an important role in mediation TNF to induce apoptosis. AIMP2 is present in full length expressed in all of exons 1 to 4, but AIMP2-DX2 (ARS interacting multi-functional protein2-Exon2 deleted), which lacks exon 2 as a splice variant, acts in opposition to AIMP2-Full length, leading to carcinogenesis when overexpressed.

Therefore, in this thesis I synthesized several compounds that selectively inhibits AIMP2DX2 without inhibiting AIMP2-Full length. In order to improve the inhibitory selectivity, activity and physical properties of the lead compound obtained from the laboratory in-house library, the structure of the lead compound was divided into three parts and various functional groups were introduced. The synthesis step was optimized by using szuki coupling, aromatic nucleophilic substitution reaction, and microwave irradiation-mediated aromatic amination reaction as key steps in the synthetic route.

The synthesized compounds were evaluated by a luciferase assay to measure the inhibition of AIMP2-Full length & AIMP2-DX02. Also, cytotoxicity of the compounds toward WI-26 & A549 cell line were evaluated and SAR (Structure-Activity Relationship) studies were performed. Finally, several novel compounds were developed having selective inhibition toward AIMP2-DX2, in submicromolar level, over AIMP2 inhibition. The selected compounds also exhibited in vitro anti-cancer activity while proved not to be toxic to normal cell lines.

Keywords: AIMP2-DX2, Anti-cancer agent, Lung cancer, Small molecule
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