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Recent advances in thymidine phosphorylase inhibitors: syntheses and prospective medicinal applications

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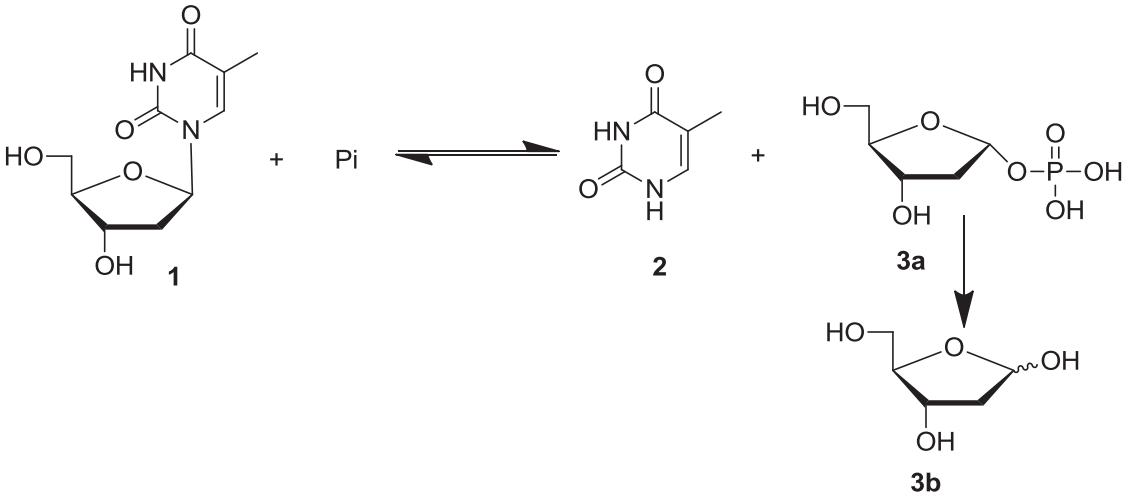
Abstract: This review paper covers recent advances in the synthetic strategies for novel TPase inhibitors and their potential medicinal applications over the last few decades. A brief introduction covering the structural aspects of TPase inhibitors is also included to facilitate understanding of diverse approaches to monitor the design of new inhibitors. TPase is an essential enzyme and its inhibition is a potential target in the development of anticancer drugs.

Key words: Thymidine phosphorylase, platelet-derived endothelial cell growth factor (PDECGF), angiogenesis, thymidine phosphorylase inhibitors, anticancer drugs, inhibitor design

1. Introduction

Thymidine phosphorylase (TPase) is an enzyme that belongs to the family of glycosyltransferases, specifically the pentosyltransferases. TPase catalyzes the cleavage of thymidine **1** (via reversible phosphorolysis) to thymine **2** and 2-deoxyribose 1-phosphate **3a**. The 2-deoxyribose 1-phosphate **3a** is further degraded to 2-deoxy-D-ribose **3b** (Scheme 1).[1,2]



Scheme 1. Reaction catalyzed by TPase.

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Recent research has found that TPase is also involved in angiogenesis, a complex process to form new blood vessels during tumor growth and metastasis.[3] Inhibition of angiogenesis within a tumor has been studied for the discovery of new cancer drugs[3,4] and it was found that 2-deoxy-D-ribose **3b** has shown chemotactic activity for endothelial cells and the angiogenic activity of TPase in vivo. In short, this monosaccharide is considered an angiogenesis-inducing factor and it has been recognized as a potential target in the improvement of anticancer drugs. TPase has been determined to be almost identical to platelet-derived endothelial cell growth factor (PDECGF) and it has been found to display antitumor activity by deactivating a variety of 5-substituted pyrimidine 2'-deoxynucleoside analogues.[5,6]

Cancerous tissue shows that TPase is overexpressed up to 10-fold in tumors as compared to normal tissue of the same organs, and so this enzyme may be an attractive cancer chemotherapy target for inhibition of tumor angiogenesis.[7,8] This enzyme is usually expressed in blood platelets and human placenta and it has been produced by different cell types in culture, such as human foreskin fibroblasts and vascular smooth muscle cells. TPase is overexpressed in several solid tumors, including carcinomas of the stomach, colon, ovary, and bladder.[9,10] Moreover TPase is highly expressed in renal carcinoma and pancreatic, breast, and lung cancer and in many chronic inflammatory diseases, such as human atherosclerosis, psoriasis, and rheumatoid arthritis.[11]

Identification of potent inhibitors of thymidine phosphorylase is very important for the treatment of different types of neoplastic and nonneoplastic diseases. Thymidine phosphorylase recognizes many nucleoside analogues that are used clinically as antiviral and/or antitumor drugs. The standard TPase inhibitors include 6-amino-5-bromouracil (6A5BU) **4** (IC_{50} = 17 μM) and 7-deazaxanthine[12] (7-DX) **5** (IC_{50} = 40 μM), which was the first purine derivative that inhibits both *E. coli* TPase and angiogenesis in a chorioallantoic membrane assay. The potent nanomolar inhibitors of human TPase are 5-chloro-6-[(2-iminopyrrolidin-1-yl)methyl]uracil hydrochloride (TPI) **6** (IC_{50} = 35 nM)[13] and 5-bromo-6-[(2-aminoimidazol-1-yl)methyl]uracil hydrochloride **7** (IC_{50} = 20 nM)[14] (Figure 1).[12–14]

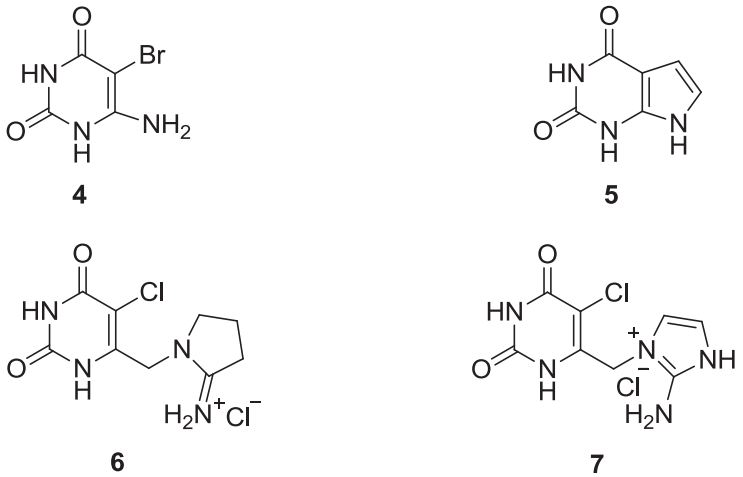


Figure 1. Potent TPase inhibitors recently described in the literature.[12–14]

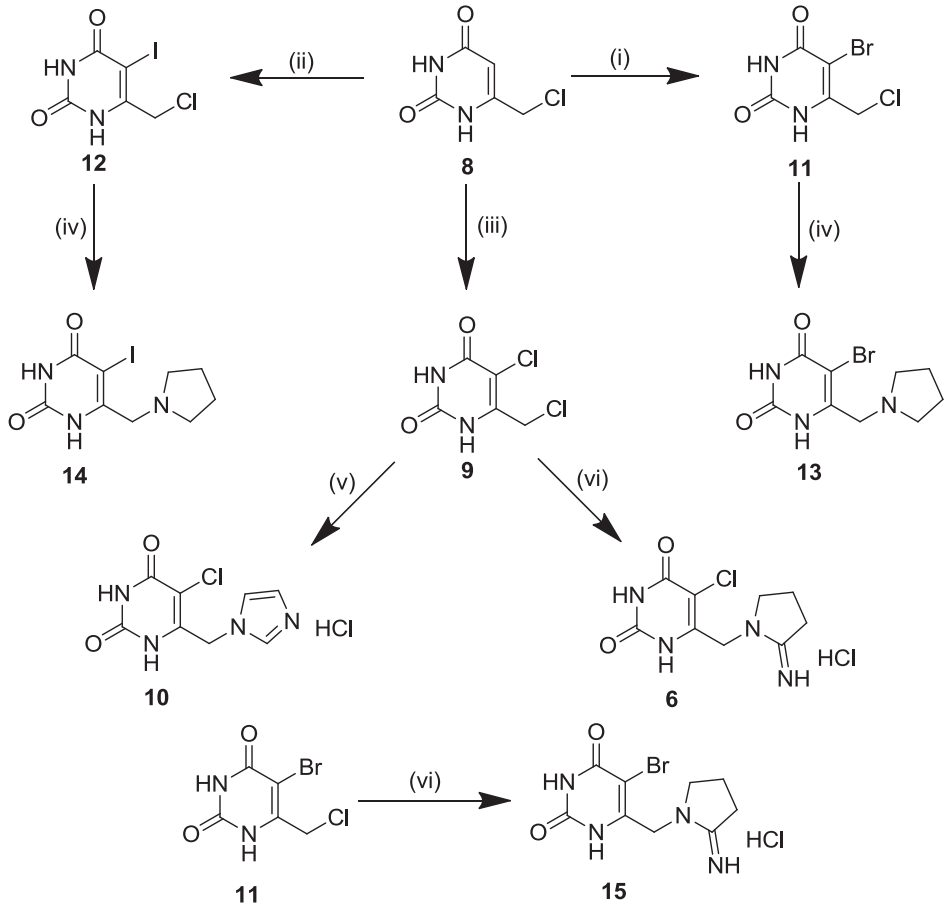
Previously this area of research has been reviewed with emphasis on enzyme inhibitory aspects.[9,10] In this review paper, we tried to cover the most recent advances in synthetic approaches for potent TPase inhibitors as well as therapeutic applications.

2. Synthesis of TPase inhibitors

There has been growing interest in TPase inhibitors in recent years, and a variety of multistep strategies have been followed to design novel and potent TPase inhibitors. Herein some of these synthetic approaches to synthesize TPase inhibitors are described in detail.

2.1. 5-Halo-6-methylene-bridge uracil derivatives

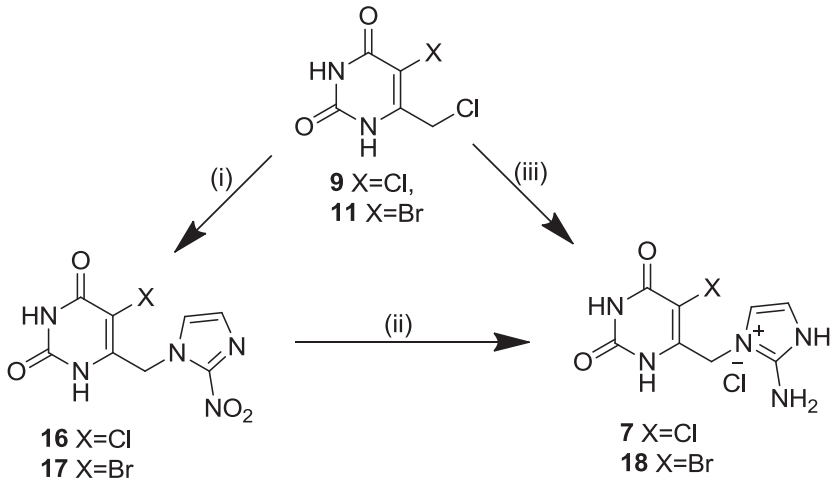
5-Chloro-6-(chloromethyl)uracil **9** was synthesized by chlorination of 6-(chloromethyl)uracil **8** with sulfuryl chloride in acetic acid in 83% yield.[15] 5-Bromo-6-(chloromethyl)uracil **11** and 6-chloromethyl-5-iodouracil **12** were prepared by halogenation of compound **8** with slight excess of *N*-bromosuccinimide (NBS) or *N*-iodosuccinimide (NIS) in DMF in 84% and 92% yields, respectively. The compounds **11** and **12** were converted to pyrrolidine analogues **13** and **14** by reacting with excess of pyrrolidine in water in 87% and 17% yields, respectively. The compound **10** was synthesized in 64% yield by heating compound **9** with acetyl imidazole in methanol.[15] The reaction of pyrrolidine-2-imine with compounds **9** and **11** furnished the 2-iminopyrrolidine derivatives **6** and **15** in 38% and 13% yields, respectively (Scheme 2).[16,17]



Scheme 2. Synthesis of 6-methylene-bridged uracil derivatives **6**, **10**, **11**, **13**, **14**, and **15**. Reagents and conditions: (i) NBS, DMF; (ii) NIS, DMF; (iii) SO_2Cl_2, AcOH; (iv) Pyrrolidine, H_2O; (v) N-acetylimidazole, MeOH; (vi) Pyrrolidin-2-imine, NaOEt, DMF.

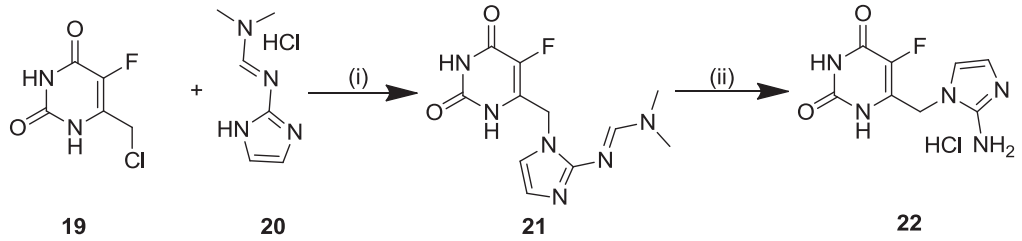
2.2. 5-Halo-6-aminoimidazolylmethyl uracil derivatives

The synthesis of compounds **9** and **11** has been previously described in the literature.[15] The prodrugs **16** and **17** were synthesized in reasonable yield (47%–78%) by coupling the 1-potassio-2-nitroimidazole with compounds **9** and **11** (Scheme 3).[10] The reduction of the nitro group in compounds **16** and **17** was achieved with Pd-C/H_2 to give the desired 5-halo-6-aminoimidazolylmethyl uracil derivatives **7** and **18** in good yield (70%–89%). The desired uracil derivatives **7** and **18** were synthesized directly from compounds **9** and **11** by condensation with 2-aminoimidazole sulfate in the presence of sodium ethoxide in DMF as a solvent in poor yields (9%–13%) (Scheme 3).[10,18]



Scheme 3. Synthesis of imidazolylmethyl uracils **7** and **18**. Reagents and conditions: (i) 1-potassio-2-nitroimidazole, DMF; (ii) 10% Pd/C, H_2, NH_3, MeOH; (iii) 2-aminoimidazole sulfate, NaOEt, DMF.

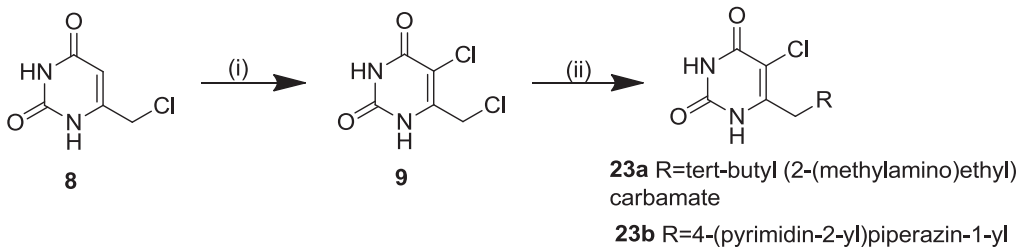
The precursor **20** was obtained by protection of 2-aminoimidazole with N,N-dimethylformamide dimethylacetal (DIEA).[19] The compound **20** on alkylation with compound **19** in DMF as a solvent gave compound **21** in 65% yield. Deprotection of **21** was performed with concentrated hydrochloric acid at 100 °C to accomplish target compound **22** in 90% yield (Scheme 4).[20,21]



Scheme 4. Synthesis of imidazolylmethyl uracil **22**. Reagents and conditions: (i) DMF, DIEA (3 equiv.), 60 °C, 12 h, argon; (ii) conc. HCl/EtOAc (1:1), 100 °C, 2 h.

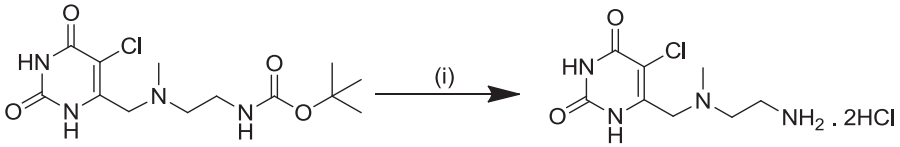
2.3. 5-Chloro-6-(dialkylaminomethyl)uracil derivatives

Commercially available 6-chloromethyluracil **8** was chlorinated through microwave-assisted chlorination with N-chlorosuccinimide (NCS) to afford 5-chloro-6-(chloromethyl)uracil **9** in 50% yield in 10 min. Reaction of compound **9** with appropriate amines under microwave irradiation at 65 °C for 15 min afforded the final compounds **23a** and **23b** in 40% and 35% yields respectively (Scheme 5).[22]



Scheme 5. Synthesis of 5-chloro-6-(dialkylaminomethyl)uracils **23a** and **23b**. Reagents and conditions: (i) NCS, DMF, MW, 55 °C; (ii) appropriate amine, MeOH, MW, 65 °C.

Compound **23a** was deprotected with HCl in dry methanol to get the primary amine derivative **24** as dihydrochloride salt in 88% yield (Scheme 6).[22]



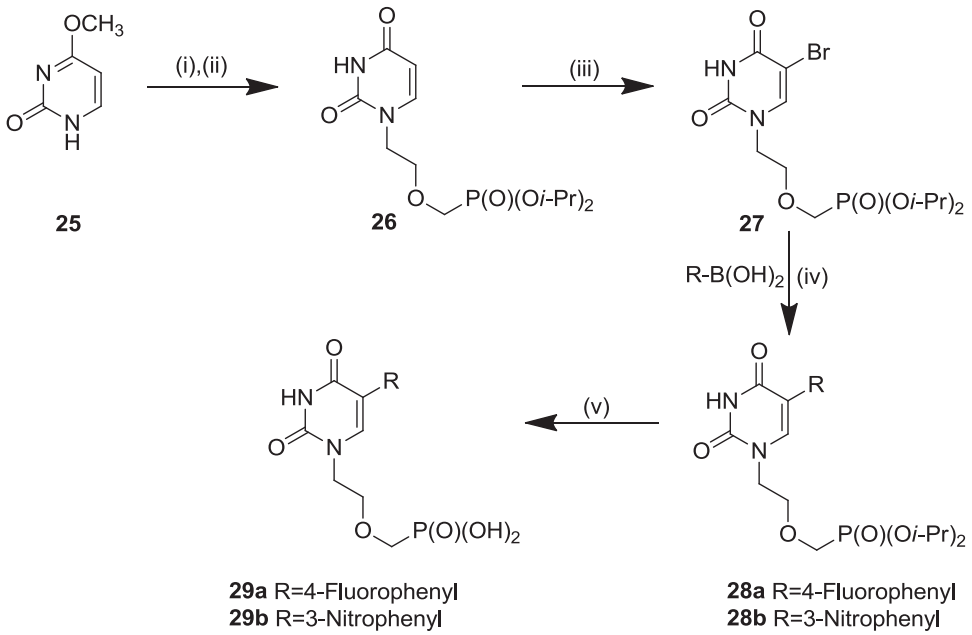
Scheme 6. Synthesis of 6-[[(2-aminoethyl)(methyl)amino]methyl]-5-chlorouracil dihydrochloride **24**. Reagents and conditions: (i) HCl, MeOH, 0–5 °C.

2.4. 5-Aryl-1-[2-(phosphonomethoxy)ethyl]uracil derivatives

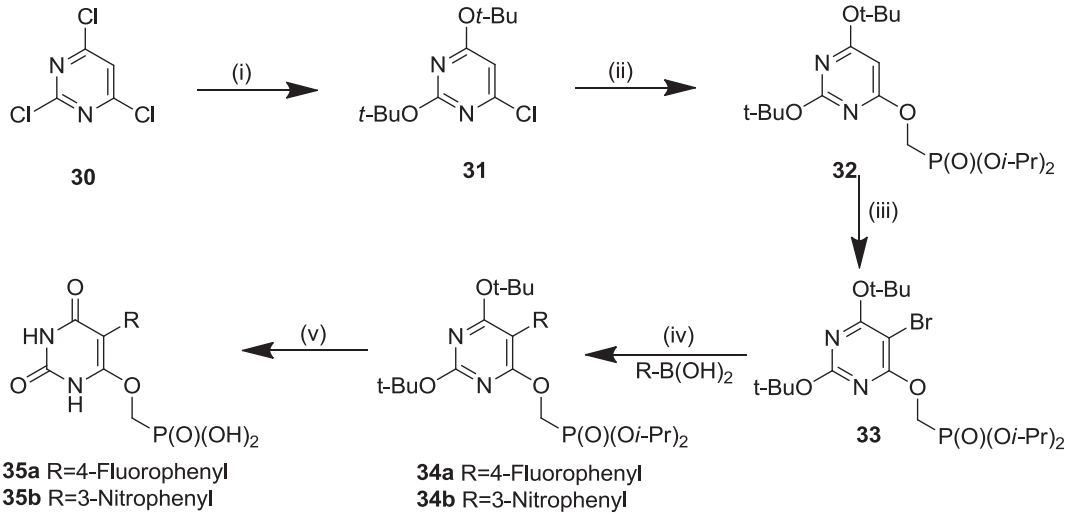
The 5-bromouracil derivative **27** was synthesized from 4-methoxypyrimidin-2-one **25** by alkylation with diisopropyl((2-chloromethoxy)methyl)phosphonate in 49% yield.[23] Further hydrolysis was carried out with an improved method[24] to afford compound **26** in 91% yield. Further bromination with *N*-bromosuccinimide (NBS) in THF in the presence of azobisisobutyronitrile (AIBN) as initiator gave compound **27** in 99% yield.[24] The Suzuki coupling of commercial aryl boronic acids with 5-bromo derivative **27** to produce pyrimidinones **28a** and **28b** both with 30% yield took place in DMF–H_2O solution catalyzed by $Pd(PPh_3)_4$ and Na_2CO_3 was used for activation of aryl boronic acids. Further reaction of compounds **28a** and **28b** with bromotrimethylsilane followed by hydrolysis gave the compounds **29a** and **29b** in 80% and 63% yields, respectively (Scheme 7).[25]

2.5. 5-Aryl-6-(phosphonomethoxy)uracil derivatives

The 5-bromo derivative **33** was synthesized from commercially available 2,4,6-trichloropyrimidine **30**, by selective protection with 2 equiv. of sodium tert-butoxide to afford compound **31** with 45% yield. Further, compound **31** reacted with isopropylhydroxymethylphosphonate in the presence of sodium hydride as a base to give phosphonate derivative **32** in 39% yield.[26] The bromination at the C-5 position of pyrimidine moiety in compound **32** was accomplished with NBS to furnish compound **33** in 99% yield (Scheme 8). The Suzuki coupling of compound **33** with aryl boronic acids to generate compounds **34a** and **34b** in 72% and 66% yields took place in the presence of DMF-H_2O solution, Na_2CO_3 as a base to activate arylboronic acids catalyzed by $Pd(PPh_3)_4$.[26] Reaction of compounds **34a** and **34b** with bromotrimethylsilane followed by hydrolysis gave compounds **35a** and **35b** in 53% and 68% yields, respectively (Scheme 8).[26]



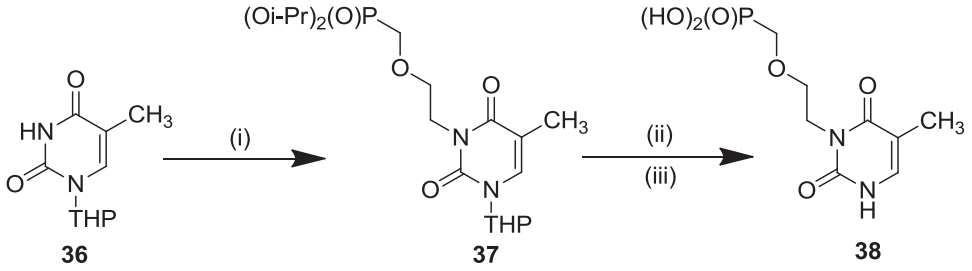
Scheme 7. Synthesis of 5-aryl-1-[2-(phosphonomethoxy)ethyl]uracils **29a** and **29b**. Reagents and conditions: (i) NaH, $CH_2ClCH_2OCH_2$-P(O)(Oi-Pr)$_2$, DMF, 80 °C; (ii) Dowex 50 (H+), 90% aq. MeOH; (iii) NBS, AIBN, THF, 60 °C; (iv) $Pd(PPh_3)_4$, Na_2CO_3, DMF, H_2O, 130 °C; (v) $(CH_3)_3SiBr$, CH_3CN, rt.



Scheme 8. Synthesis of 5-aryl-6-(phosphonomethoxy)uracils **35a** and **35b**. Reagents and conditions: (i) t-BuONa (2 equiv), THF, 0 °C to reflux; (ii) NaH, $HOCH_2P(O)(Oi\text{-}Pr)_2$, THF, 0 °C to rt; (iii) NBS, AIBN, THF, 60 °C; (iv) $Pd(PPh_3)_4$ (0.1 equiv.), Na_2CO_3, DMF, H_2O, 130 °C; (v) $(CH_3)_3SiBr$, CH_3CN, rt.

2.6. 5-Methyl-3-[2-(phosphonomethoxy)ethyl]uracil derivative

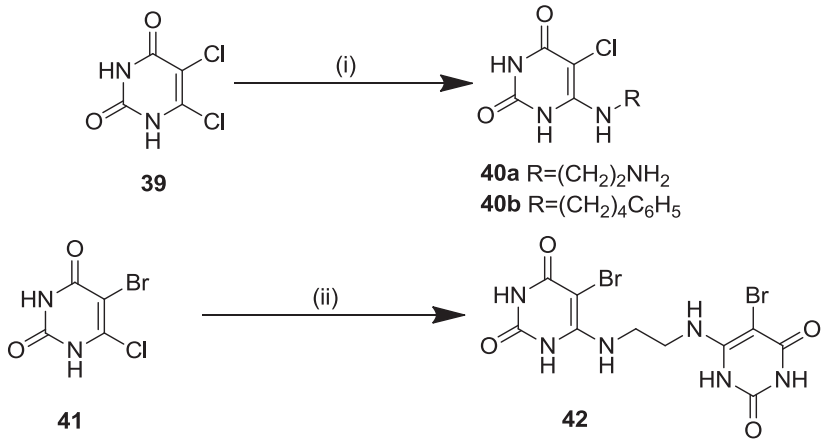
The selective N^3-alkylation of 1-(tetrahydro-pyran-2-yl)thymine **36** with diisopropyl((2-chloroethoxy)methyl) phosphonate took place in good preparative yield in the presence of sodium hydride in dimethylformamide to afford **37** (Scheme 9);[27] further deprotection of the phosphonoalkyl group was carried out with bromotrimethylsilane in MeCN and tetrahydropyran (THP) was deprotected by trifluoroacetic acid in water to obtain final product **38** in 32% yield (Scheme 9).[27]



Scheme 9. Synthesis of 5-methyl-3-[2-(phosphonomethoxy)ethyl]uracil **38**. Reagents and conditions: (i) diisopropyl((2-chloroethoxy)methyl)phosphonate, NaH, DMF, 100 °C; (ii) Me_3SiBr, MeCN, rt; (iii) CF_3COOH, H_2O, reflux.

2.7. 5-Halo-6-(aminoalkyl)uracil derivatives

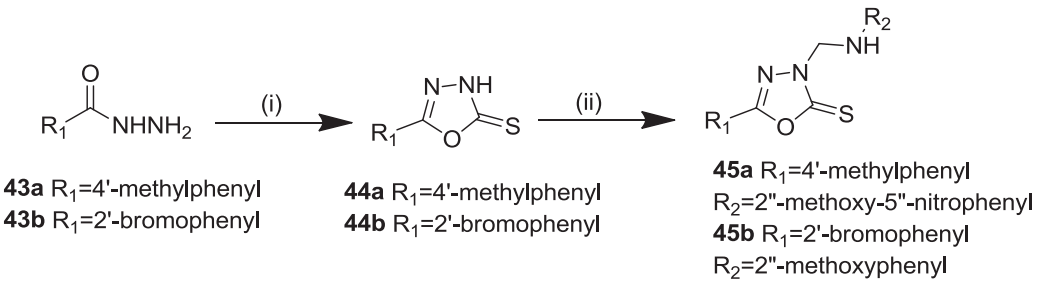
The compounds **40a** and **40b** were synthesized by the reaction of 5,6-dichlorouracil derivative **39** with excess of the selective amines at 100 °C without solvent for 1–4 h.[28,29] The compound **42** was prepared by reaction of 5-bromo-6-chlorouracil **41** with 1,2-diaminoethane in ethanol at room temperature for 14 h (Scheme 10).[30]



Scheme 10. Synthesis of 5,6-disubstituted uracil **40a**, **40b**, and bis-uracil **42**. Reagents and conditions: (i) R-NH_2, 100 °C, 1–4 h; (ii) 1,2-diaminoethane, EtOH.

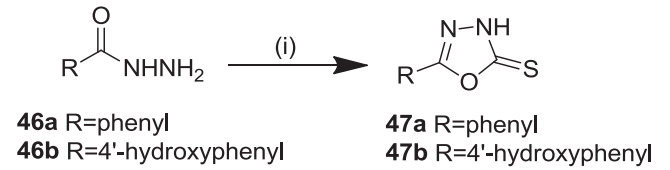
2.8. 1,3,4-Oxadiazole-2-thione derivatives

The 5-substituted 1,3,4-oxadiazoline-2-thiones **44a** and **44b** precursors were synthesized by the reaction of acid hydrazides **43a** and **43b** with carbon disulfide in KOH under microwave irradiation.[31] The Mannich bases **45a** and **45b** were synthesized by reaction of compounds **44a** and **44b** with formalin and primary amines in 43% and 94% yields, respectively (Scheme 11).[32]



Scheme 11. Synthesis of 1,3,4-oxadiazoline-2-thione derivatives **45a** and **45b**. Reagents and conditions: (i) KOH/Al_2O_3, CS_2, MW; (ii) HCHO, EtOH, R_2-NH_2.

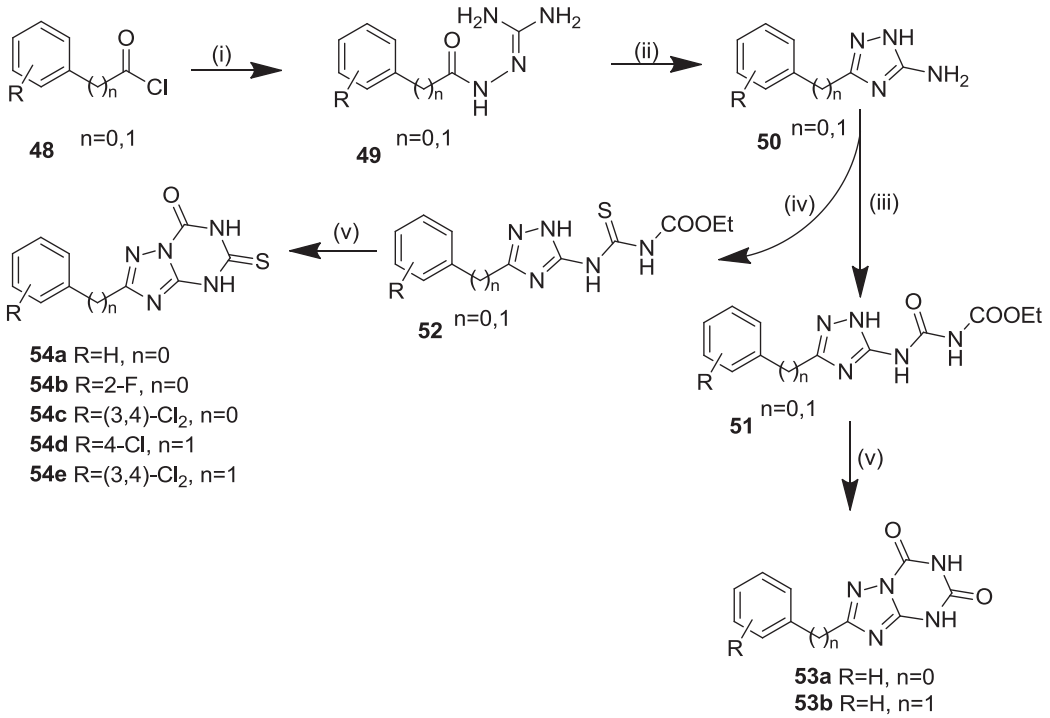
1,3,4-Oxadiazoline-2-thione derivatives **47a** and **47b** were synthesized by condensing respective hydrazides **46a** and **46b** with carbon disulfide in potassium hydroxide and ethanol on alumina and the reaction proceeded and completed efficiently under microwave irradiation within 7 min in 90% and 91% yields, respectively (Scheme 12).[33]



Scheme 12. Synthesis of 1,3,4-oxadiazole-2-thione derivatives **47a** and **47b**. Reagents and conditions: (i) KOH/EtOH, CS_2, MW.

2.9. 1,2,4-Triazolo[1,5-a][1,3,5]triazin-5,7-dione and its 5-thioxo derivatives

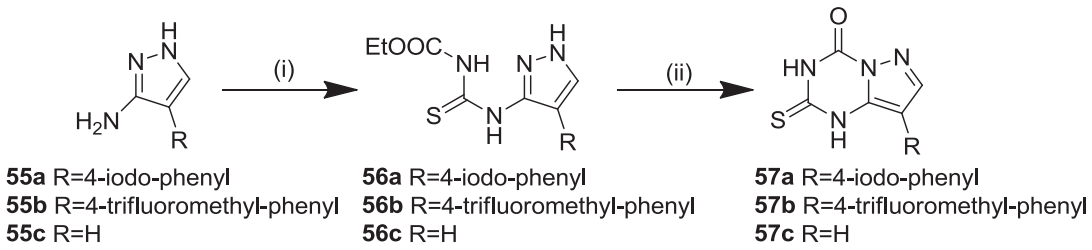
The synthesis of amidoguanidines **49** from commercially available acid chlorides **48**, followed by microwave-assisted cyclocondensation in water affords 5-amino-1,2,4-triazoles **50**. Further 5-amino-1,2,4-triazoles **50** on reaction with ethyl isocyanoformate or ethoxycarbonyl isothiocyanate in the presence of DMF afforded the urea **51** and thiourea **52** derivatives. These derivatives (**51** and **52**) undergo intramolecular heterocyclization in the presence of base, resulting in the formation of target compounds **53a**, **53b**, and **54a**–**54e** in good yields (47%–78%) within 20 min (Scheme 13).[34,35]



Scheme 13. Synthesis of 1,2,4-triazolo[1,5-a][1,3,5]triazin-5,7-dione and its 5-thioxo derivatives **53a**, **53b**, and **54a-54e**. Reagents and conditions: (i) aminoguanidine hydrochloride, fusion at 180 °C, NaOH; (ii) water, MW irradiation, 180 °C; (iii) ethyl-isocyano formate, DMF, rt; (iv) ethoxycarbonyl isothiocyanate, DMF, rt; (v) NaOH, ethanol (80%), 100 °C, 20 min.

2.10. 1,3-Dihydro-pyrazolo[1,5-a][1,3,5]triazin-2-thioxo-4-one derivatives

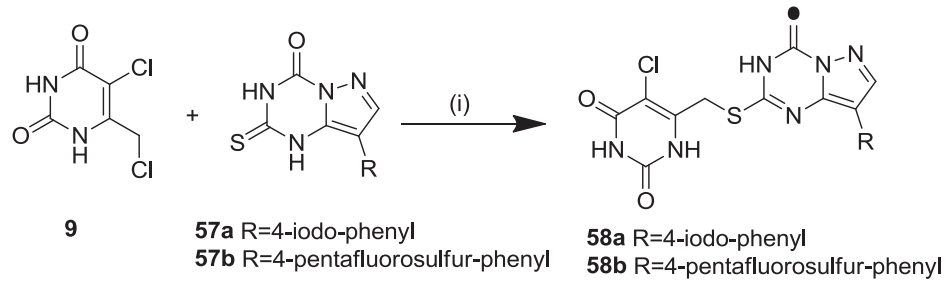
The *N*-ethoxycarbonyl-*N*′-(pyrazol-3-yl)thiourea derivatives **56a**–**56c** were synthesized in quantitative yields by reaction of amines **55a**–**55c** with ethoxycarbonyl isothiocyanate at room temperature in anhydrous DMF (Scheme 14). The target compounds **57a**–**57c** were synthesized in good yields (54%–86%) by intramolecular ring annulation reaction of compounds **56a**–**56c** in the presence of sodium ethoxide as a catalyst (Scheme 14).[36,37]



Scheme 14. Synthesis of 1,3-dihydro-pyrazolo[1,5-a][1,3,5]triazin-2-thioxo-4-ones **57a**–**c**. Reagents and conditions: (i) ethoxycarbonyl isothiocyanate, DMF, rt; (ii) EtONa, ethanol, reflux.

2.11. 3*H*-2-(5-chlorouracil-6-methylthio)-pyrazolo[1,5-a]-1,3,5-triazin-4-one derivatives

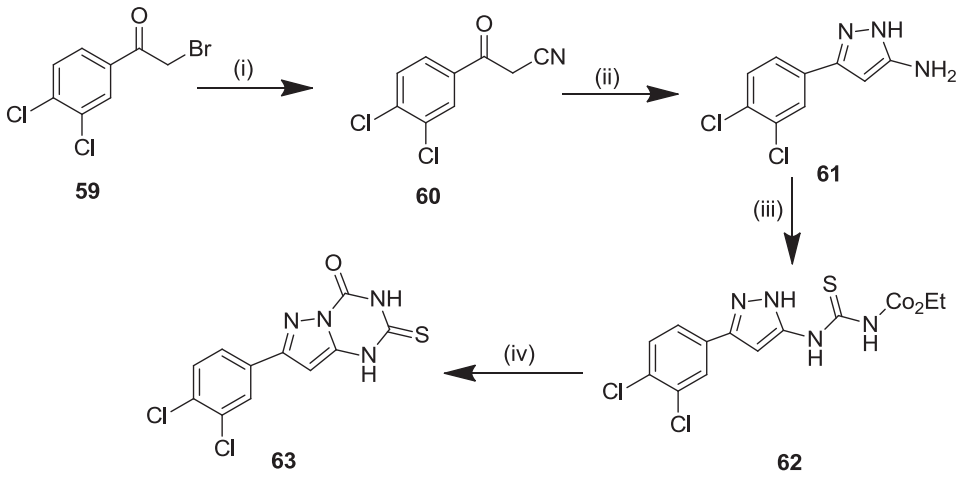
The target compounds **58a** and **58b** were synthesized in 89% and 67% yields, respectively, by refluxing pyrazolo[1,5-a]-1,3,5-triazine-2-thioxo-4-one **57a**, **57b**, and 5-chloro-6-chloromethyluracil **9** in a mixture of sodium ethoxide and methanol for 0.5 h (Scheme 15).[38]



Scheme 15. Synthesis of 3*H*-2-(5-chlorouracil-6-methylthio)-pyrazolo[1,5-a]-1,3,5-triazin-4-ones **58a** and **58b**. Reagents and conditions: (i) EtONa, MeOH, reflux.

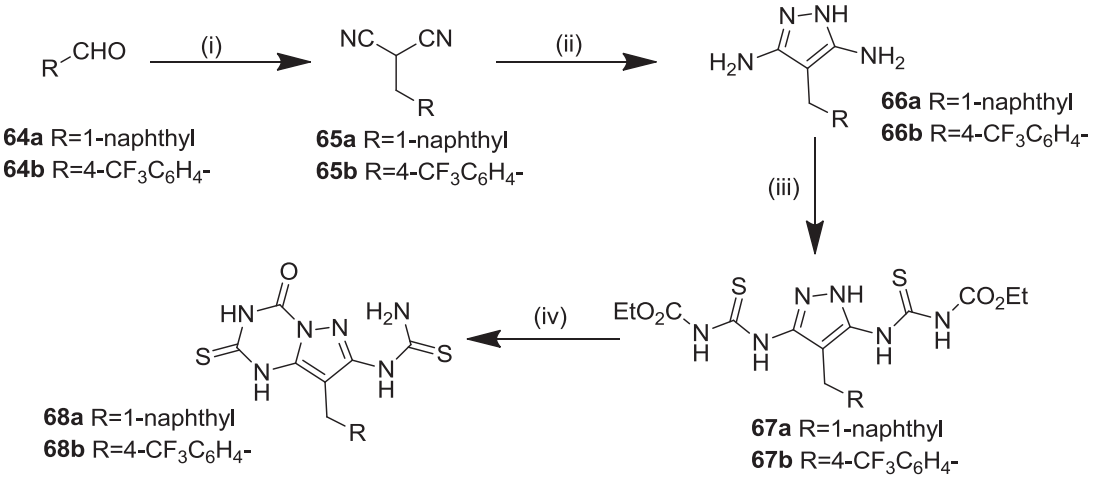
2.12. 2-Thioxo-pyrazolo[1,5-a][1,3,5]triazin-4-one derivatives

The cyanoacetophenone **60** was synthesized from the reactions of commercially available substituted bromoacetophenone **59** with sodium cyanide. The compound **61** was furnished by cyclocondensation reaction upon treatment with hydrazine hydrate under microwave irradiation (Scheme 16).[39] The reaction of aminopyrazole **61** with ethoxycarbonyl isothiocyanate in DMF afforded the thiourea derivative **62**. The final compound **63** was formed in 63% yield by intramolecular heterocyclization of compound **62** in the presence of base (Scheme 16).[40]



Scheme 16. Synthesis of 2-thioxo-pyrazolo[1,5-a][1,3,5]triazin-4-one **63**. Reagents and conditions: (i) NaCN, HCl, aqueous-ethanol, rt; (ii) $NH_2NH_2.H_2O$, methanol, MW, 140 °C, 1 h (iii) ethoxycarbonyl isothiocyanate, DMF, rt, 5 h (iv) NaOH, ethanol (80%), 100 °C, 20 min.

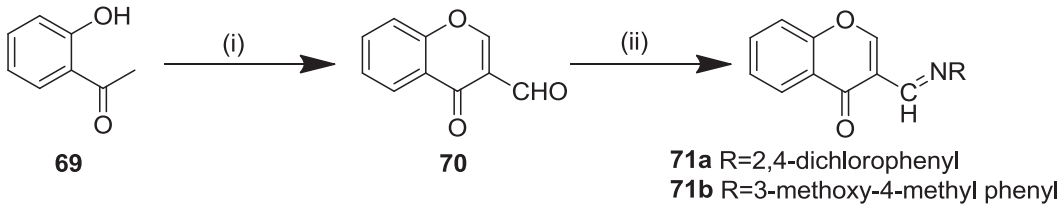
The intermediates **65a** and **65b** were formed by reductive alkylation of malononitrile with benzaldehydes **64a** and **64b**,[41] which on reaction with hydrazine hydrate in refluxing ethanol yielded the corresponding 3,5-diaminopyrazoles **66a** and **66b** (Scheme 17). The thiourea derivatives **67a** and **67b** were furnished by the reaction of **66a** and **66b** with ethoxycarbonyl isothiocyanate in DMF. The target compounds **68a** and **68b** were formed by intramolecular heterocyclization of **67a** and **67b** in the presence of base in 62% and 68% yields, respectively (Scheme 17).[42]



Scheme 17. Synthesis of 2-thioxo-pyrazolo[1,5-a][1,3,5]triazin-4-one derivatives **68a** and **68b**. Reagents and conditions: (i) malononitrile, ethanol (aq.), $NaBH_4$, 1.0 M HCl, rt; (ii) $NH_2NH_2.H_2O$, Ethanol, reflux, 5–8 h; (iii) Ethoxycarbonyl isothiocyanate, DMF, rt, 5 h; (iv) NaOH, ethanol (80%), 100 °C, 20 min.

2.13. Schiff bases of 3-formylchromone

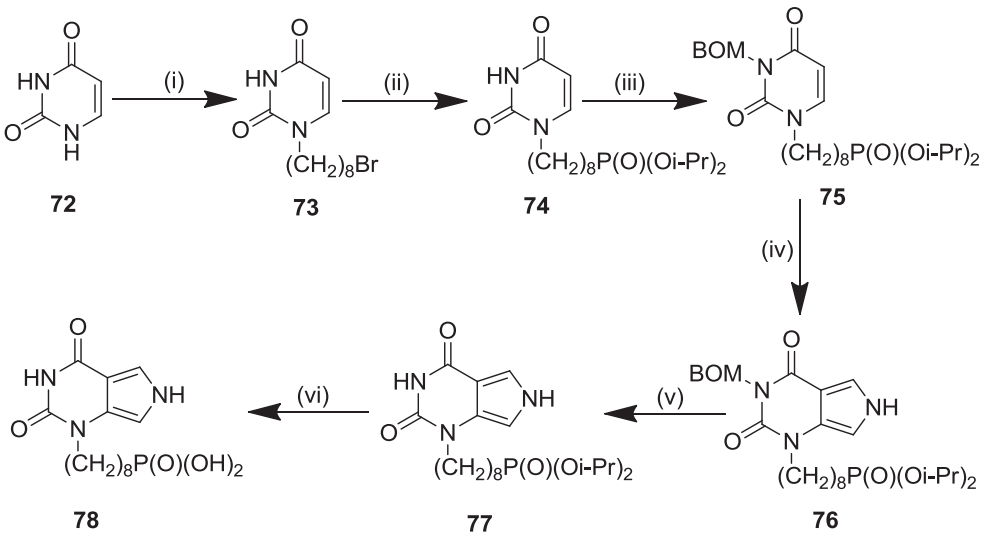
3-Formylchromone **70** was synthesized by the Vilsmeier–Haack formylation.[43] The Schiff bases **71a** and **71b** were synthesized by condensation reaction of 3-formylchromone **70** with aromatic amines in ethanol in 52% and 72% yields, respectively (Scheme 18).[44]



Scheme 18. Synthesis of Schiff bases of 3-formyl chromone **71a** and **71b**. Reagents and conditions: (i) anhydrous DMF, $POCl_3$; (ii) R-NH_2, EtOH.

2.14. 8-Aza-7,9-dideazaxanthine

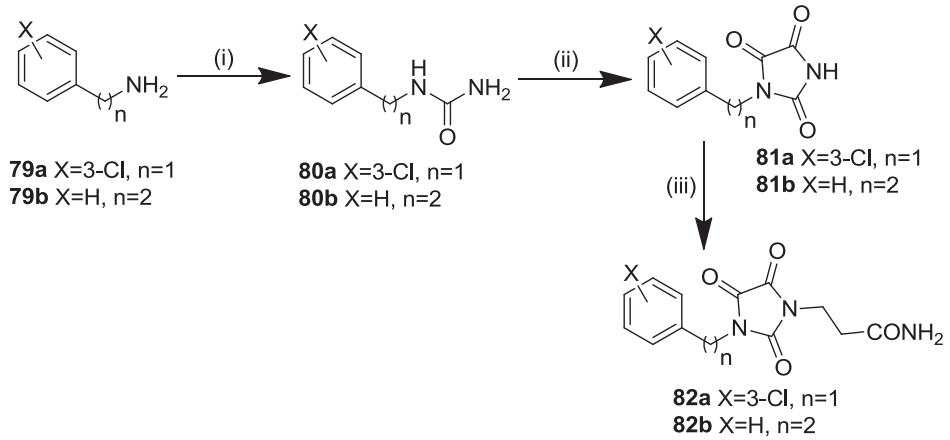
The 1-(8-bromooctyluracil) **73** was prepared in 75% yield by the alkylation of compound **72** using 1,8-dibromooctane. The phosphonate **74** was synthesized in 76% yield by utilizing the Michaelis–Arbuzov reaction from compound **73**. The compound **74** was protected with benzyloxymethylacetal (BOM) to obtain **75** in 60% yield. The compound **75** was reacted with toluenesulfonylmethyl isocyanide (TosMIC) under Van Leusen pyrrole synthesis conditions to afford protected 8-aza-7,9-dideazaxanthine **76** in 46% yield (Scheme 19).[12,45] Catalytic hydrogenation of **76** produced compound **77** in 85% yield. The final compound 8-aza-7,9-dideazaxanthine nucleotide **78** was obtained in 43% yield by the deprotection of compound **77** using trimethylsilyl bromide (TMSBr) (Scheme 19).[12,45]



Scheme 19. Synthesis of 8-aza-7,9-dideazaxanthine **78**. Reagents and conditions: (i) BSA, 1,8-dibromooctane, CH_3CN, 75 °C, 3 h; (ii) P(Oi-Pr)$_3$, 160 °C, 6 h; (iii) NaH, BOM-Cl, DMF, 75 °C, 5 h; (iv) TosMIC, DMSO-dioxane (1:4), 75 °C, 6 h; (v) H_2/Pd, MeOH, overnight; (vi) Me_3SiBr, CH_3CN, overnight then NH_3/H_2O.

2.15. 2,4,5-Trioxoimidazolidin-1-yl derivatives

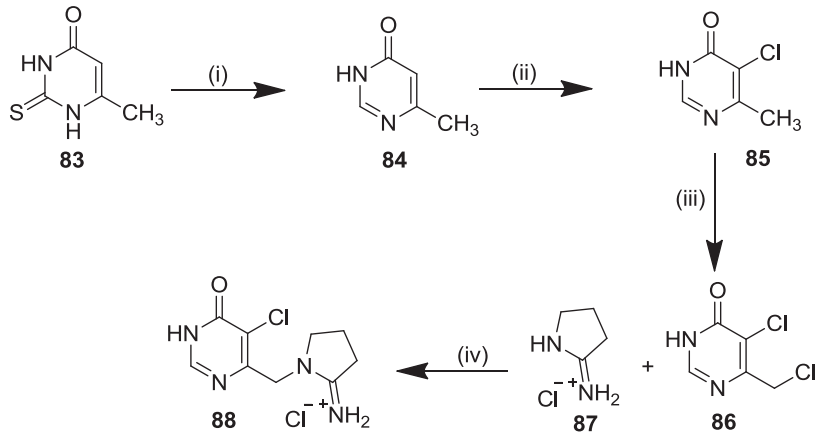
The *N*-(benzyl)urea derivatives **80a** and **80b** were synthesized by the reaction of benzylamine derivatives **79a** and **79b** with urea in the presence of acid in good yields. The reaction of *N*-(benzyl)urea derivatives **80a** and **80b** with oxalyl chloride gave the 1-(benzyl)-imidazolidine-2,4,5-triones **81a** and **81b** in good yields.[46] The novel amide analogues **82a** and **82b** were accomplished in 38% and 96% yields, respectively, by reaction of imidazolidine-2,4,5-trione derivatives **81a** and **81b** with 3- bromopropionamide (Scheme 20).[47]



Scheme 20. Synthesis of 2,4,5-trioxoimidazolidin-1-yl derivatives **82a** and **82b**. Reagents and conditions: (i) urea, HCl, heat; (ii) oxalyl chloride, THF; (iii) 3-bromopropionamide, KOH, EtOH, heat.

2.16. 5-Chloro-6-[(2-iminopyrrolidin-1-yl)methyl]-3H-pyrimidin-4-one hydrochlorid prodrug

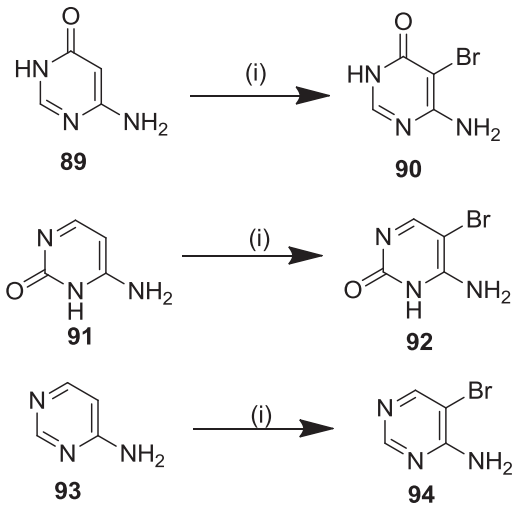
The synthesis of 6-methyl-3H-pyrimidin-4-one **84** was accomplished by desulphurization reaction of 6-methyl-2-thiouracil **83** by hydrogenolysis in the presence of alkaline solution of Raney nickel.[48] The chlorination of compound **84** at C-5 position with *N*-chlorosuccinimide (NCS) in acetic acid afforded compound **85** in 73.7% yield.[49] 5-Chloro-6-(chloromethyl)-3H-pyrimidin-4-one **86** was synthesized in 10.2% yield by radical halogenation using benzoyl peroxide and NCS.[50] Subsequent nucleophilic substitution of compound **86** with 2-iminopyrrolidine hydrochloride **87** furnished prodrug **88** in 38.1% yield (Scheme 21).[50]



Scheme 21. Synthesis of 5-chloro-6-[(2-iminopyrrolidin-1-yl)methyl]-3Hpyrimidin-4-one hydrochloride **88**. Reagents and conditions: (i) Raney Ni/H_2, NH_3, H_2O; (ii) NCS, AcOH; (iii) NCS, $(PhCO_2)_2$, CCl_4; (iv) NaOEt, DMF.

2.17. XO-activated prodrugs of 6-amino-5-bromouracil

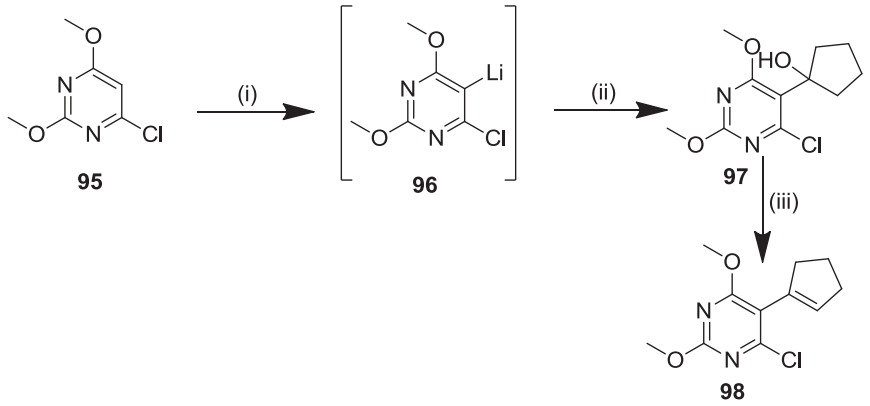
6-Amino-5-bromopyrimidine xanthine oxidase (XO) activated prodrugs of 6A5BU **90**, **92**, and **94** were synthesized by electrophilic substitution of the suitable 6-aminopyrimidine with molecular bromine in 47%, 56%, and 53% yields, respectively (Scheme 22).[51]



Scheme 22. Synthesis of XO-activated prodrugs of 6A5BU **90**, **92**, and **94**. Reagents and conditions: (i) Br_2.

2.18. 2,4-Dimethoxy-6-chloro-5-cyclopent-1-en-1-yluracil derivative

The intermediate **96** was synthesized from commercially available 4-chloro-2,6-dimethoxypyrimidine **95** by direct ortho-lithiation with butyllithium at –78 °C. Intermediate alcohol **97** was prepared simply by quenching the lithiated reaction mixture with cyclopentanone monitoring by a slow rise in temperature. The final compound **98** was formed in 49% yield by refluxing compound **97** with hydrochloric acid in THF/dioxane (Scheme 23).[52]

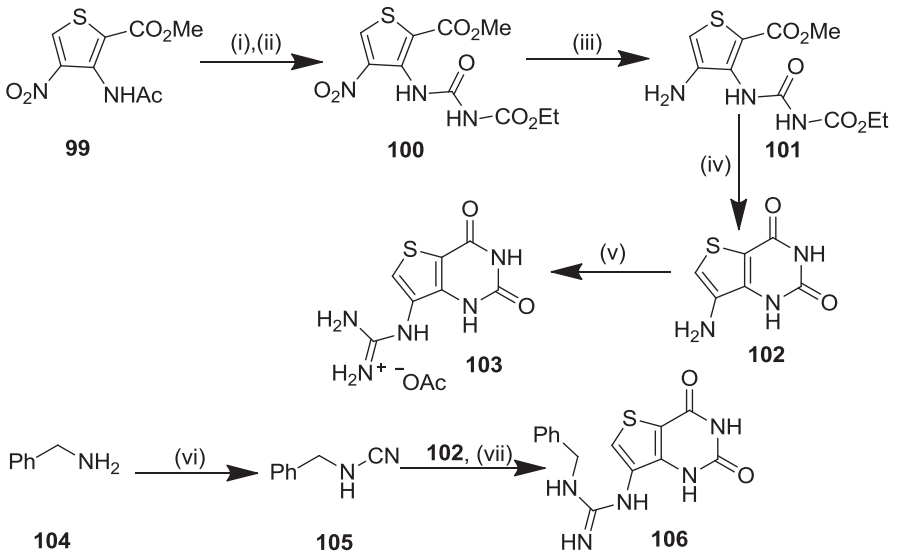


Scheme 23. Synthesis of 6-chloro-5-cyclopent-1-en-1-yluracil **98**. Reagents and conditions: (i) n-BuLi, THF, –78 °C; (ii) cyclopentanone, –78 °C to rt; (iii) conc. HCl, THF, dioxane, reflux.

2.19. *N*-(2,4-dioxo-1,2,3,4-tetrahydro-thieno[3,2-d]pyrimidin-7-yl)guanidine derivative

The thiophene regioisomer **99** with nitro moiety was prepared according to the literature procedure reported [53] by Elliott et al. The acetyl group was detached with HCl in methanol, and the resulting free amine was reacted with ethyl isocyanatoformate to furnish compound **100** in 87% yield. Reduction of nitro groups with iron in refluxing acetic acid in the presence of ethanol afforded amine **101** in 72% yields. Subsequent cyclization of **101** using sodium methoxide in methanol produced thienopyrimidine dione **102** in 86% yield (Scheme 24). The desired guanidine salt **103** with 52% yield was obtained by the reaction of compound **102** with cyanamide in acetic acid at 110 °C.[54] The benzyl amine **104** was converted to cyanamide **105** in 72% yield by the reaction of

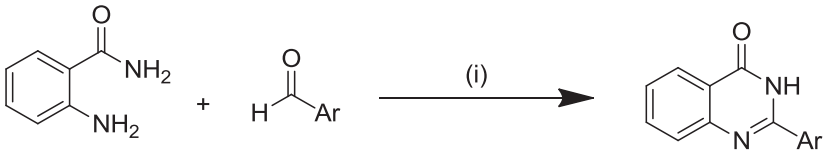
cyanogen bromide and sodium bicarbonate in methanol. The target compound guanidine **106** was synthesized in 13% yield by the reaction of thienopyrimidine dione **102** and cyanamide **105** in hexafluoroisopropanol (HFIP) at 100 °C for 65 h in a sealed tube (Scheme 24).[55]



Scheme 24. Synthesis of N-(2,4-dioxo-1,2,3,4-tetrahydro-thieno[3,2-d]pyrimidin-7-yl)guanidines **103** and **106**. Reagents and conditions: (i) HCl, MeOH, heat; (ii) ethyl isocyanatoformate, $CHCl_3$, heat; (iii) Fe, AcOH, EtOH, heat; (iv) NaOMe, MeOH, heat; (v) cyanamide, AcOH, heat; (vi) cyanogen bromide, $NaHCO_3$, MeOH, 0 °C; (vii) HFIP, 100 °C.

2.20. 2-Arylquinazolin-4(*3H*)-one derivatives

The compounds **109a**, **109b**, and **109c** were synthesized by reaction of anthranilamide with various benzaldehyde derivatives with 89%, 88%, and 97% yields, respectively. The reaction was catalyzed by $CuCl_2.2H_2O$ in ethanol under reflux (Scheme 25).[56]



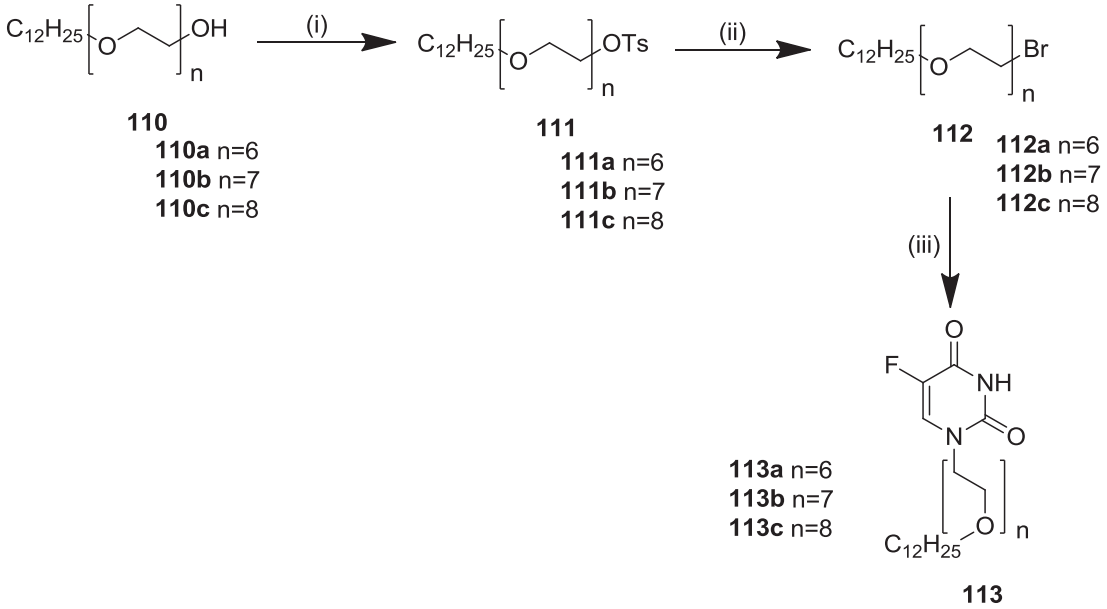
109a Ar=4-methylphenyl
109b Ar=3-hydroxyphenyl
109c Ar=phenyl

Scheme 25. Synthesis of 2-arylquinazolin-4(3H)-ones **109a**, **109b**, and **109c**. Reagents and conditions: (i) $CuCl_2.2H_2O$, EtOH, reflux.

2.21. 5-Fluorouracil derivatives

The commercially available compounds **110a**, **110b**, and **110c** on reaction with TsCl in THF as solvent in the presence of NaH gave derivatives **111a**, **111b**, and **111c**,[57] which on further reaction with NaBr in acetone at 65 °C furnished bromo derivatives **112a**, **112b**, and **112c**. The bromo derivatives **112a**, **112b**, and **112c** on

reaction with 5 equivalents of 5-fluorouracil (5-FU) in the presence of a stoichiometric amount of K_2CO_3 in DMF gave 5-FU derivatives **113a**, **113b**, and **113c** (Scheme 26).[57]



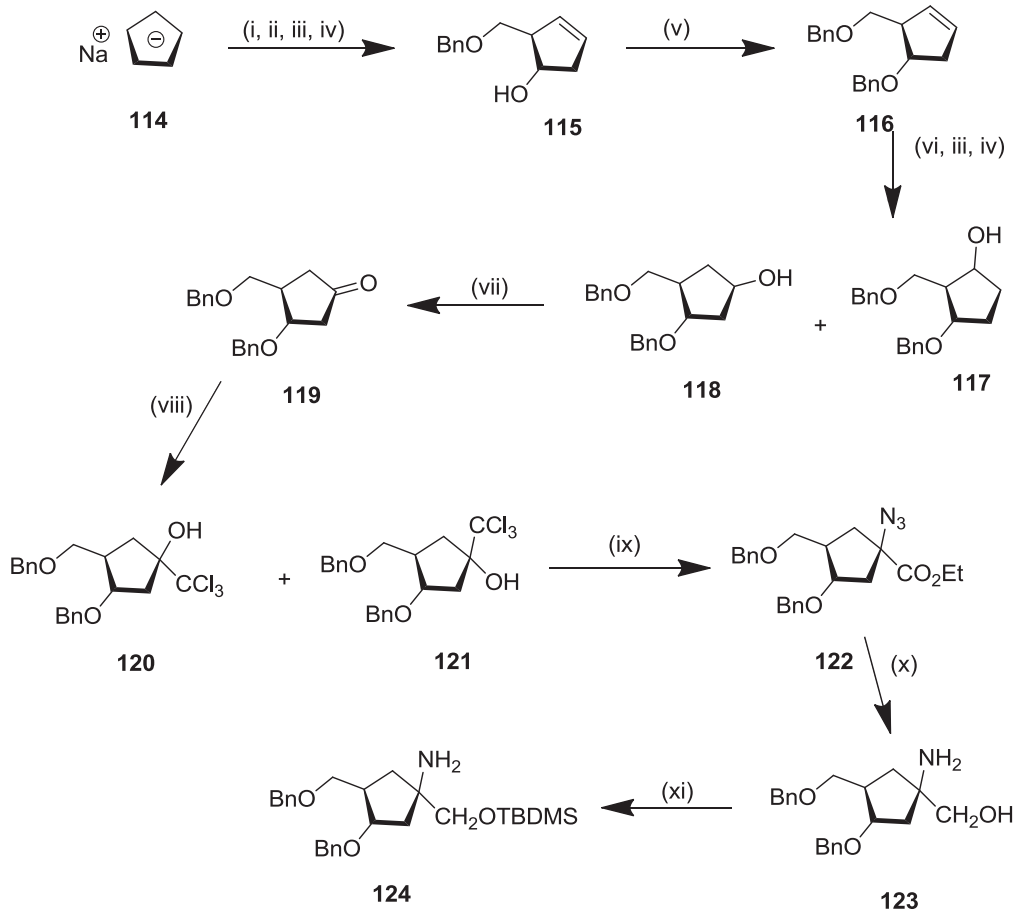
Scheme 26. Synthesis of 5-fluorouracil (5-FU) derivatives **113a**, **113b**, and **113c**. Reagents and conditions: (i) NaH, TsCl, THF; (ii) NaBr, acetone; (iii) 5-FU, K_2CO_3, DMF.

2.22. Transition state analogues of thymidine phosphorylase

The compound **115** was obtained from sodium cyclopentadienylide **114** in 45% yield.[58] The alkene intermediate **115** was benzylated to get compound **116** in 75% yield and then hydroborated using borane to afford a mixture of alcohols **117** and **118** in 10% and 77% yield, respectively (Scheme 27);[59] both were separated by chromatography. The oxidation of alcohol **118** with PDC afforded the compound **119** in 82% yield. The reaction of **119** with trichloromethylcarbanion gave compounds **120** and **121** in 15% and 47% yield, respectively. Then reaction of NaN_3 and DBU with compound **121** gave azido ethyl ester **122** in 89% yield, which on further reaction with LAH gave amino alcohol **123** in 100% yield. Treatment of amino alcohol **123** with TBDMSCl under standard conditions gave silyl ether **124** in 64% yield (Scheme 27).[59]

The thymidine moiety was achieved by the reaction of **18** with 3-methoxy-2-methylacryloyl isocyanate to afford intermediate **125** in 85% yield. Then reaction with HCl and 1,4-dioxane to achieve cyclization and desilylation gave **126** in 79% yield over the two steps. Deprotection of the benzyl group was carried out to get **127** with 86% yield (Scheme 28).[60] The compound **127** was used as a source for compound **129** and **135**. Treatment of compound **127** with diisopropylphosphoramidite gave the tetrabenzyl compound **128** in 79% yield, which on hydrogenolysis afforded the putative transition state analogue target phosphate **129** in 86% yield (Scheme 28).[60] The *N*-protection of compound **127** with BOMCl gave **130** in 72% yield, followed by oxidation of **130** with Dess–Martin periodinane to get **131** in 80% yield. The anion of diethyl phosphate was generated in situ with sodium hydride and added to the aldehyde **131**, and then the intermediate alcohol was converted to protected phosphonate **133** in 51% yield over two steps using a Barton–McCombie deoxygenation

procedure.[61] The *N*-deprotection of compound **133** gave **134** in 78% yield, which on further deprotection of benzyl group afforded compound **135** in 90% yield (Scheme 28).[60]

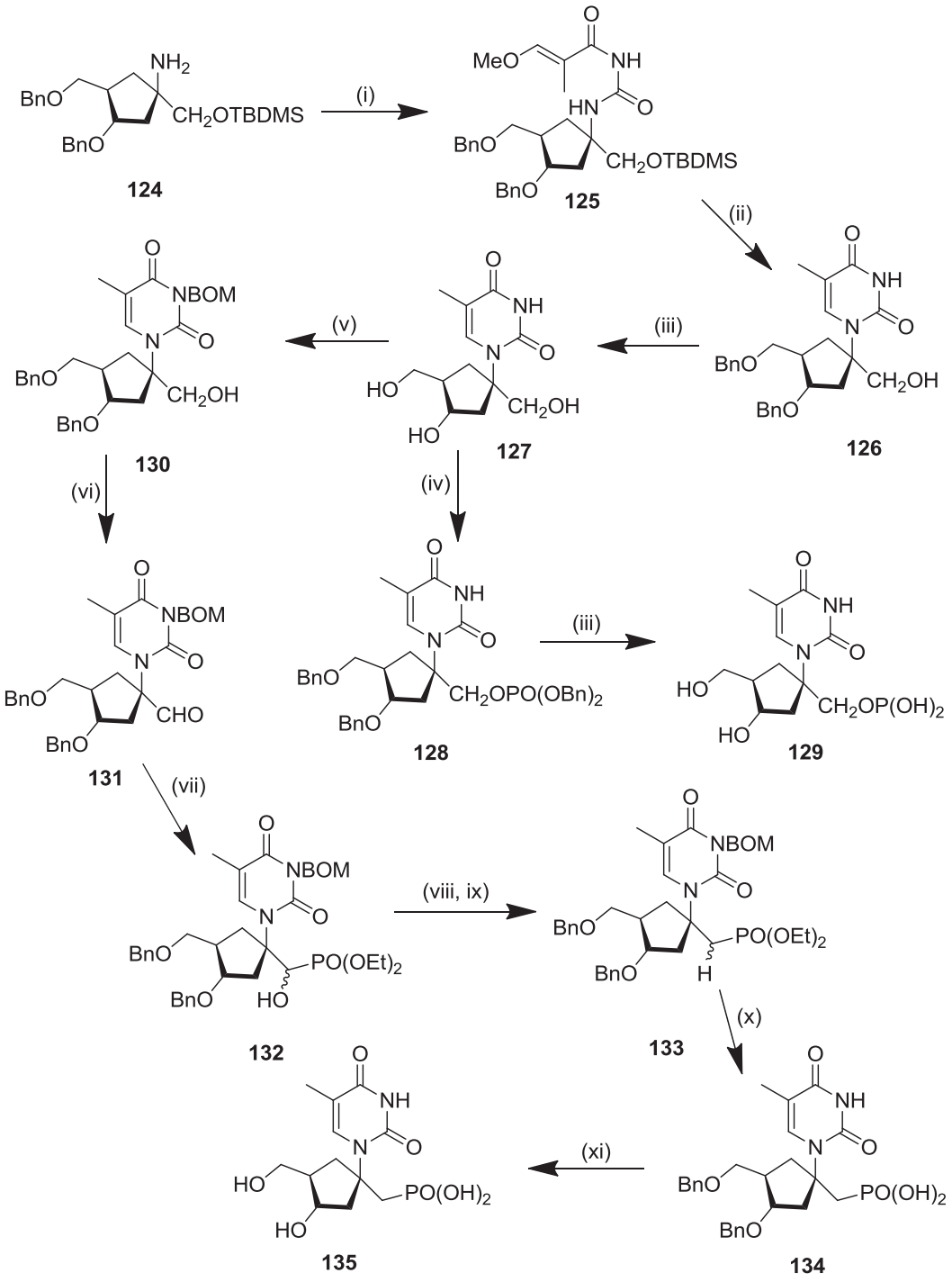


Scheme 27. Synthesis of compound **124**. Reagents and conditions: (i) BOMCl, THF –60 °C to 20 °C; (ii) (-)-diisopinan-3-yl borane, THF, –60 °C to 20 °C; (iii) 3 M NaOH, 0 °C; (iv) 30% H_2O_2, THF, 0 °C to rt; (v) BnBr, NaH, DMF, 0 °C to rt; (vi) 9-BBN, THF, 0 °C to rt; (vii) PDC, Ac_2O, CH_2Cl_2, rt; (viii) $CHCl_3$, LHMDS, THF, –78 °C to rt; (ix) NaN_3, DBU, EtOH, 50 °C; (x) LAH, THF, 0 °C to rt; (xi) TBDMSCl, imidazole, DMF, rt.

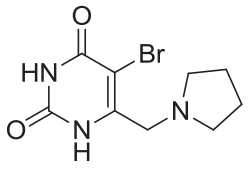
3. Biological activities

Yano et al.[15] synthesized a series of 5-halo-6-methyline–bridged uracil derivatives, among which 5-bromo-6-(pyrrolidinylmethyl)uracil **13** was the most active compound with IC_{50} = 0.51 μM on human TPase. The reference compound was 6A5CU with IC_{50} = 15 μM (Figure 2).

Fukushima et al.[14] and Yano et al.[17] prepared a series of 5-halo-6-methylene–bridged uracil derivatives and found that 5-chloro-6-[(2-iminopyrrolidinyl)methyl]uracil hydrochloride **6** with IC_{50} = 0.035 μM on human TPase was the most active compound. In silico docking showed the 2-iminopyrrolidinyl group matching well at the active site of human TPase. Additionally exhibited improved plasma concentration of F_3dThd in monkeys and on oral administration to mice potentiate in vivo antitumor activity of F_3dThd (Figure 3).

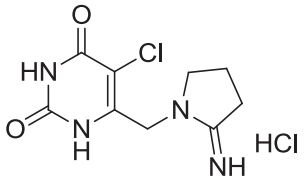


Scheme 28. Synthesis of compounds **129** and **135**. Reagents and conditions: (i) $MeOCH{=}C(CH_3)CONCO$, MeCN, rt; (ii) 10% HCl, 1,4-dioxane, reflux; (iii) H_2 (1 atm), Pd/C, EtOH, rt; (iv) dibenzyl diisopropylphosphoramidite, tetrazole, CH_2Cl_2, rt; (v) BOMCl, DBU, DMF, rt; (vi) Dess–Martin periodinane, CH_2Cl_2, rt; (vii) diethyl phosphate, NaH, THF, 0 °C to rt; (viii) CS_2, NaH, MeI, DMF, rt; (ix) nBu_3SnH, benzene, reflux; (x) TMSBr, Et_3N, CH_2Cl_2, rt; (xi) BBr_3, CH_2Cl_2, Ar, –78 °C.



13

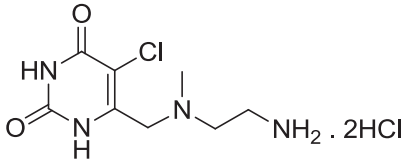
Figure 2. Structure of compound **13**.[15]



6

Figure 3. Structure of compound **6**.[14,17]

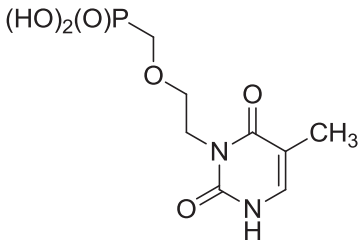
Corelli et al.[22] synthesized a series of 5-chloro-6-(dialkylaminomethyl) uracil derivatives. Among the synthesized compounds, 6-[[(2-aminoethyl)(methyl)amino]methyl]-5-chlorouracil dihydrochloride **24** was found to be most active, having IC_{50} = 0.83 μM on human TPase (Figure 4).



24

Figure 4. Structure of compound **24**.[22]

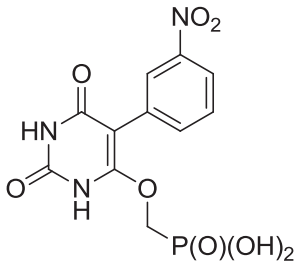
Pomeisl et al.[27] reported that 5-methyl-3-[2-(phosphonomethoxy)ethyl]uracil **38** having inhibitory efficacy of 2-deoxythymidine at concentration 100 μM expressed in V_i/V_o (V_i = rate of phosphorolysis in the presence of inhibitors, V_0 = rate of phosphorolysis in the absence of inhibitors) is 0.82 on human placenta (Figure 5).



38

Figure 5. Structure of compound **38**.[27]

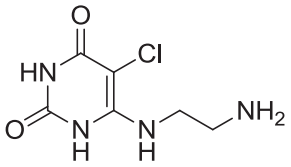
Pomeisl et al.[26] reported a series of 5-aryl-6-(phosphonomethoxy) uracil derivatives; among them 5-(3-nitrophenyl)-6-(phosphonomethoxy) uracil **35b** was most active, having inhibitory activity against human TPase with IC_{50} = 2.43 $\pm$ 0.33. Herein the increase in inhibitory activity of compound **35b** towards human TPase was due to incorporation of the phosphonoalkyl group at C-6 position of 5-aryluracil moiety (Figure 6).



35b

Figure 6. Structure of compound **35b**.[26]

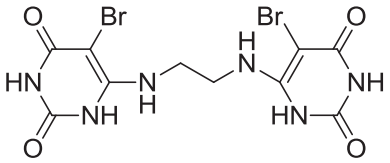
Klein et al.[29] reported a series of 5,6-disubstituted uracil derivatives; among them compound **40a** (IC_{50} = 0.25 μM) was most active due to halogen at C-5 along with strong basic moiety at C-6 position of uracil moiety (Figure 7).



40a

Figure 7. Structure of compound **40a**.[29]

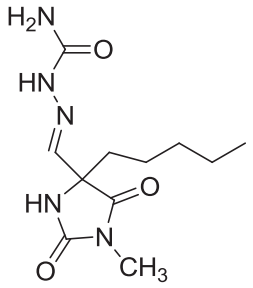
Nencka et al.[30] reported a series of 5,6-disubsituted bis-uracil derivatives; among them bis-uracil derivative **42** exhibited IC_{50} = 3.6 ± 0.3 μM. Compound **42** is more active as compared to the standard compound 6A5CU with IC_{50} = 15 μM. In inhibitory activity of compound **42** the role of second uracil moiety is not completely understood but could enhance the probability of interaction with the enzyme (Figure 8).



42

Figure 8. Structure of compound **42**.[30]

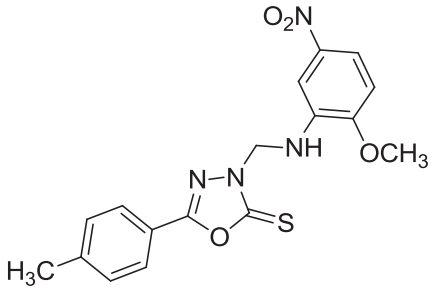
McNally et al.[62] reported the in silico screening of many compounds to identify a novel class of inhibitor of human and *Escherichia coli* TPase; among them 2-[(1-methyl-2,5-dioxo-4-pentyl-4-imidazolidinyl)methylene]semicarbazide **136** was found to inhibit *E. coli* TPase and human TPase with IC_{50} = 20 μM and IC_{50} = 77 μM, respectively (Figure 9).



136

Figure 9. Structure of compound **136**.[62]

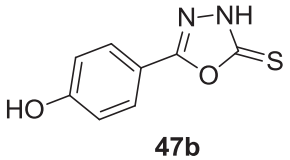
Shahzad et al.[32] synthesized a series of Mannich bases of 1,3,4-oxadiazoline-2-thiones. Among the synthesized compounds **45a** was found to be the most active due to the presence of a nitro group with IC_{50} = 14.40 ± 2.45 μM (standard IC_{50} = 39.28 ± 0.76 μM). An in silico docking study showed the formation of H-bonding with Arg171 and Ser186 (Figure 10).



45a

Figure 10. Structure of compound **45a**.[32]

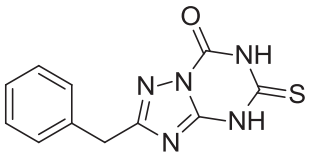
More recently, Shahzad et al.[33] reported a series of 5-substituted-1,3,4-oxadiazole-2-thione derivatives by microwave irradiation. Herein 5-(4-hydroxyphenyl)-1,3,4-oxadiazole-2-thione derivative **47b** having IC_{50} = 38.24 ± 1.28 μM against thymidine phosphorylase was found to be the most active compound (Figure 11).



47b

Figure 11. Structure of compound **47b**.[33]

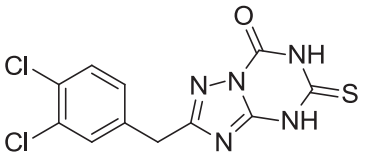
Bera et al.[35] reported a series of 5-thioxo-[1,2,4]triazolo[1,5-a][1,3,5]triazin-7-one derivatives; among them 3-benzyl-1,2,4-triazolo[1,5-a][1,3,5]triazin-5-thio-7-one **54a** (IC_{50} = 39.56 ± 1.76 μM) was the most active as it showed different binding orientations and proved a mixed mode of inhibition. The reference compound showed IC_{50} = 39.28 ± 0.76 μM (Figure 12).



54a

Figure 12. Structure of compound **54a**.[35]

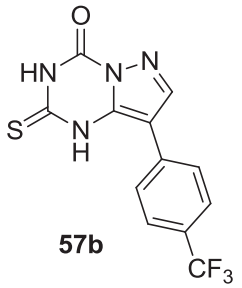
Bera et al.[34] synthesized a series of 5-thioxo-[1,2,4]triazolo[1,5-a][1,3,5]triazin-7-one derivatives and found that substitute-1,2,4-triazolo[1,5-a][1,3,5]triazin-5-thio-7-one **54e** ($IC_{50} = 2.95 \pm 0.74$ μM) was the most active among the tested series due to the dichlorophenyl unit as compared to reference compound 7-deazaxanthine ($IC_{50} = 39.28 \pm 0.76$ μM) (Figure 13).



54e

Figure 13. Structure of compound **54e**.[34]

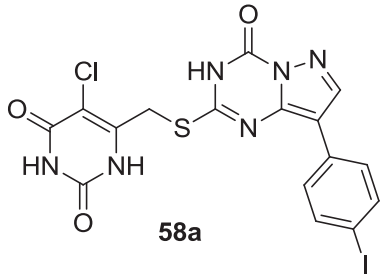
Sun et al.[37] reported a series of pyrazolo[1,5-a][1,3,5]triazin derivatives; among them substitute-1,3-dihydro-pyrazolo[1,5-a][1,3,5]triazin-2-thioxo-4-one **57b** ($IC_{50} = 0.040 \pm 0.005$ μM) was the most active due to the more electron-withdrawing group at C-4 position of the phenyl ring, which makes it a noncompetitive inhibitor. The reference compound 7-deazaxanthine showed $IC_{50} = 39.28 \pm 0.76$ μM (Figure 14).



57b

Figure 14. Structure of compound **57b**.[37]

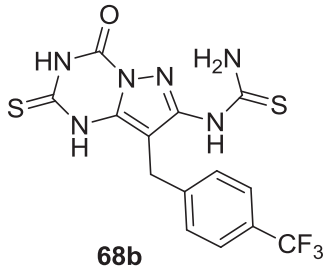
Sun et al.[38] synthesized a range of uracil derivatives and found that substitute-3H-2-(5-chlorouracil-6-methylthio)-pyrazolo[1,5-a]-1,3,5-triazin-4-one **58a** having $IC_{50} = 0.36 \pm 0.10$ μM was the most potent compound among the tested compounds (Figure 15).



58a

Figure 15. Structure of compound **58a**.[38]

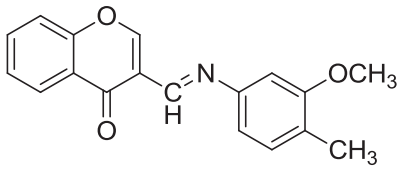
Bera et al.[42] reported a library of 2-thioxo-pyrazolo[1,5-a][1,3,5]triazin-4-one derivatives and found that substitute-2-thioxo-pyrazolo[1,5-a][1,3,5]triazin-4-one **68b** having IC_{50} = 3.82 μM was the most active compound. In silico docking studies showed a hydrogen bonding interaction of **68b** with six different amino acids, whereas the standard 7-deazaxanthine showed IC $_{50}$ = 39.28 ± 0.76 μM (Figure 16).



68b

Figure 16. Structure of compound **68b**.[42]

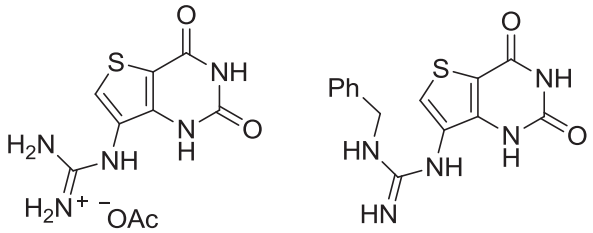
Khan et al.[44] reported a series of Schiff bases of 3-formyl chromone. Among them the compound **71b** with IC_{50} = 19.77 μM was found to be the most potent compound in comparison to the standard 7-deazaxanthine with IC $_{50}$ = 39.28 ± 0.76 μM (Figure 17).



71b

Figure 17. Structure of compound **71b**.[44]

Price et al.[54] synthesized many N-(2,4-dioxo-1,2,3,4-tetrahydro-thieno[3,2-d]pyrimidin-7-yl)guanidine derivatives and tested their activity against human TPase enzyme. Compounds **103** and **106** were found to be the most active, with K_i = 76 ± 6.4 and 64.3 ± 1 μM, respectively (Figure 18).



103 **106**

Figure 18. Structures of compounds **103** and **106**.[54]

Javaid et al.[56,63] reported a series of 2-arylquinazolin-4(3H)-one derivatives among which compounds **3a** and **3b** were found to be most active, with IC_{50} = 42.9 ± 1.0 μM and IC_{50} = 59.5 ± 1.9 μM, respectively. The standard compound used was 7-deazaxanthine with IC_{50}= 41.0 ± 1.63 μM (Figure 19).

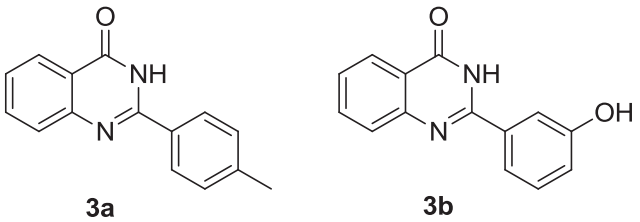


Figure 19. Structures of compounds **3a** and **3b**.[56,63]

Petaccia et al.[64] reported the 5-FU derivatives **7a**, **7b**, and **7c** and carried out docking studies on them and found that the enzymatic phosphorolysis of 0.10 mM thymidine was reduced 38%, 40%, and 43% by compounds **7a**, **7b**, and **7c**, respectively (Figure 20).

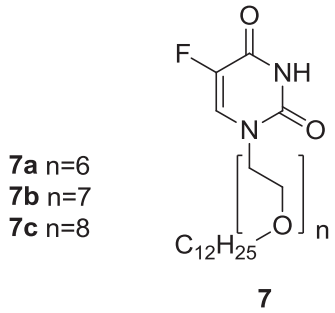


Figure 20. Structures of compounds **7a**, **7b**, and **7c**.[64]

Murray et al.[65] synthesized a range of C-6-uracil substituted derivatives. Among the synthesized compounds, 1-[(5-chloro-2,4-dihydroxypyrimidin-6-yl)methyl]pyridinium chloride **137** (IC_{50} = 0.23 μM) was recognized as the best inhibitor and found to be 5-fold more potent than the known inhibitor, 6-amino-5-bromouracil (IC_{50} = 1.10 μM) (Figure 21).

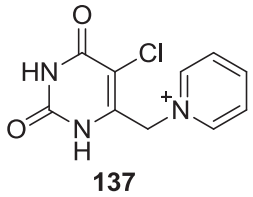


Figure 21. Structure of compound **137**.[65]

Nencka et al.[52] reported a series of 5-substituted-6-chlorouracil derivatives and found that substitution at the C-5 position by certain hydrophobic groups shows significant inhibitory activity against TPase. 2,4-Dimethoxy-6-chloro-5-cyclopent-1-en-1-yluracil **98** was found to be the most potent derivative among the synthesized series with K_i = 0.20 $\pm$ 0.03 μM for thymidine phosphorylase enzyme (purified from placenta) expressed in V79 cells with K_i = 0.29 $\pm$ 0.04 μM (Figure 22).

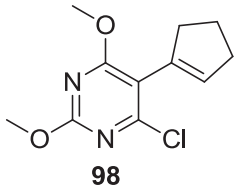
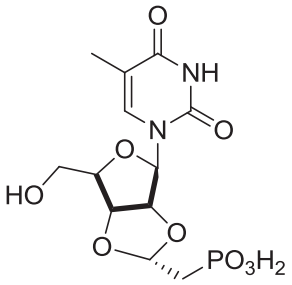


Figure 22. Structure of compound **98**.[52]

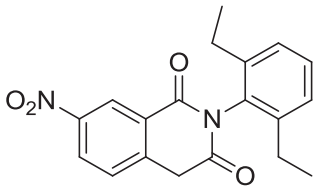
Allan et al.[66] reported a range of novel, multisubstrate, bicyclic pyrimidine nucleoside inhibitors of human thymidine phosphorylase and found that the orientation of the phosphonate moiety was critical for inhibitory activity. Among the synthesized compounds, **138** was the most active with $K_i = 0.236 \pm 0.007$ μM against human TPase (Figure 23).



138

Figure 23. Structure of compound **138**.[66]

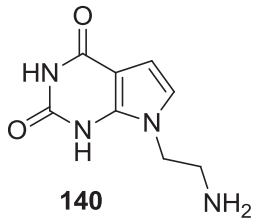
Kita et al.[67] synthesized several *N*-phenylhomophthalimide derivatives and found that 2-(2,6-diethylphenyl)-7-nitro-1,2,3,4-tetrahydroisoquinoline-1,3-dione **139** was the most potent inhibitor, having two binding sites for TPase, with $IC_{50} = 246$ μM against human TPase (Figure 24).



139

Figure 24. Structure of compound **139**.[67]

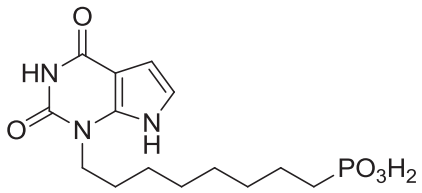
Hirota et al.[68] synthesized a series of 7-deazaxanthine derivatives. Among the synthesized compounds 7-(2-aminoethyl)-deazaxanthine **140** was found to be most active, with $IC_{50} = 44$ μM against TPase enzyme (Figure 25).



140

Figure 25. Structure of compound **140**.[68]

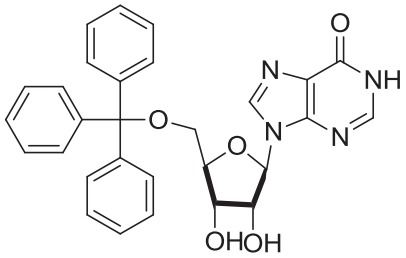
Liekens et al.[69] synthesized a series of prototype inhibitors in which the compound 9-(8-phosphonooctyl)-7-deazaxanthine (TP65) **141** at 250 μM completely inhibited TPase-induced formation of microvascular sprouts from endothelial cell aggregates in a three-dimensional fibrin gel (Figure 26).



141

Figure 26. Structure of compound **141**.[69]

Liekens et al.[70–72] synthesized a series of novel 5'-O-trityl nucleoside derivatives. Among the series, 5'-O-trityl-inosine (KIN59) **142** was found to be the most active compound and allosterically inhibits TPase enzymatic activity. It inhibits recombinant bacterial (*E. coli*) with IC_{50} = 44 μM and human TPase with IC_{50} = 67 μM (Figure 27).



142

Figure 27. Structure of compound **142**.[70–72]

4. Conclusion

In this review, the most important synthetic strategy for thymidine phosphorylase inhibitors was revised comprehensively. Attention to TPase inhibitors has been considerably transformed in the last decade and different synthetic approaches have been followed to design novel inhibitors. However, in our view, there is still opportunity for additional improvement, and in the following years the development of new compounds, particularly exploring leads different from the traditional 6-substituted uracil derivatives, will arise. Innovative perspectives have also been freshly opened for the improvement of new compounds. Herein the literature of the past few decades has been covered about key synthetic approaches to TPase inhibitors. Further, TPase inhibitory activity of most effective compounds was described in terms of IC_{50}, percentage inhibition, and inhibitory constant (K_i). This review is expected to be useful for the scientific community who are involved in developing potent thymidine phosphorylase inhibitors.

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