Approaches towards better immunosuppressive agents

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Abstract

Several classes of compounds are applied in clinics due to their immunosuppressive properties in transplantology and treatment of autoimmune diseases. Derivatives of mycophenolic acid, corticosteroids and chemotherapeutics bearing heterocyclic moieties like methotrexate, azathioprine, mizoribine, ruxolitinib are active substances with investigated mechanism of action. However, improved synthetic approaches of known drugs and novel derivatives are still being reported to attempt better accessibility and therapeutic properties. In this review article, we present synthesis of the designed chemical structures based on recent literature reports concerning novel compounds as promising immunosuppressive drugs. Moreover, some of the discussed derivers revealed also other types of activities with prospective medicinal potential.

Keywords: mycophenolic acid; corticosteroids; heterocycles; immunosuppressive agents, anti-proliferative activity, inhibitors

1. Introduction

Although transplantology is one of the most important treatment methods, the prevention of rejection of transplanted organs similarly to the cure of autoimmune diseases requires immunosuppressive therapy. Despite the significant choice in clinically applied active substances, there are led broad studies to achieve enhanced efficacy and reduced side effects [1-5]. In this review, we focus on types of chemical compounds possessing immunosuppressive properties in terms of their structures, synthetic issues, mechanism of action, and therapeutic usage. Apart from modification of approved drugs, there are also reported newly developed compounds based on heterocyclic derivatives. Although immunosuppressants' functions concern mainly human immune system responses, their activities may also indicate significant features against cancer cell lines or antiviral, antibacterial potencies. The discussed compounds were chosen according to their significant role in medicine. However, their structural modifications are still investigated to improve their therapeutic properties and extension of clinical applications.

2. Mycophenolic acid derivatives

Mycophenolic acid (MPA) **1** (Fig. **1**) possesses immunosuppressive activity as an uncompetitive inhibitor of inosine-5'-monophosphate dehydrogenase (IMPDH), the enzyme catalyzing the nicotinamide adenine dinucleotide (NAD)-dependent oxidation of inosine-5'-monophosphate (IMP) to xanthine 5'-monophosphate (XMP), which is needed for the growth T and B cells. According to these properties, MPA derivatives: sodium mycophenolate (MPS) **1** and mycophenolate mofetil (2-morfolinoethyl, MMF) **3**, are used in clinics for autoimmune disorders treatment and prophylaxis of solid organ rejection as Myfortic (Novartis) and CellCept (Roche), respectively [6-8]. In clinics, they can be used with calcineurin inhibitors or corticosteroids [1-3].
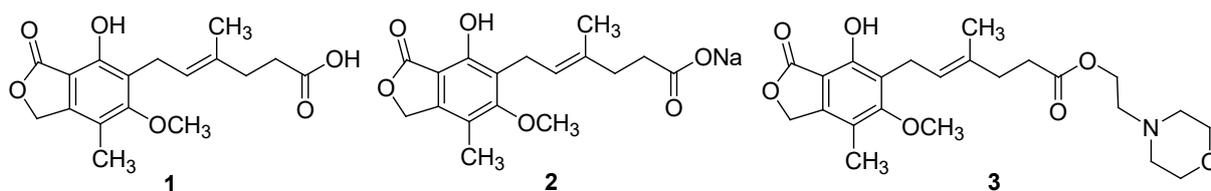
Fig. (1). Mycophenolic acid **1** and its prodrugs **2** and **3**.

Despite the progress in immunosuppressive therapy, the risk of graft rejection has not been eliminated so far and applied drugs also cause side-effects [4-5 Therefore, new potential immunosuppressants are being investigated, including structurally modified analogs of mycophenolic acid (MPA) [9-17]. According to reported data, some structural factors are crucial for the maintenance of antiproliferative activity of MPA derivatives, like the free phenolic group (interactions with Thr 333, Glu 441 of IMPDH) and polar (carboxylic) group at the end of the side chain of MPA (interactions with Ser 276 of IMPDH) [9].

IMPDH enzyme exists in the two isoforms possessing 514 amino acid residues and molecular mass 56 kDa. IMPDH I and IMPDH II are of identical size and share 84% sequence identity. Isoform I is expressed in both normal and neoplastic cells, while type II expression is preferentially up-regulated in human neoplastic cell lines [9]. Both IMPDH I and IMPDH II constitute targets for immunosuppressive agents, where MPA indicates a slightly higher activity towards isoform II. MPA and its derivatives also exhibit anticancer activity [18-23].

Shah and Kharkar reported direct synthesis of novel *N*-alkyl or *N*-aryl MPA amides from MPA **1** and respective amines as IMPDH II inhibitors possessing anticancer activity *in vitro* [24]. Some of designed compounds revealed comparable to parent MPA **1** enzyme inhibition and significant activity against cancer cell lines. Noteworthy, *N*-2-methylbenzyl **4** and *N*-4-phenylbutyl **5** mycophenolic acid amides (Fig. **2**) gave lower half maximal inhibitory concentration (IC50) against human breast adenocarcinoma (MDA-MB-231), prostate cancer (DU145), and glioblastoma astrocytoma (U87MG) cell lines in comparison to both, MPA and cisplatin, and similar to doxorubicin.
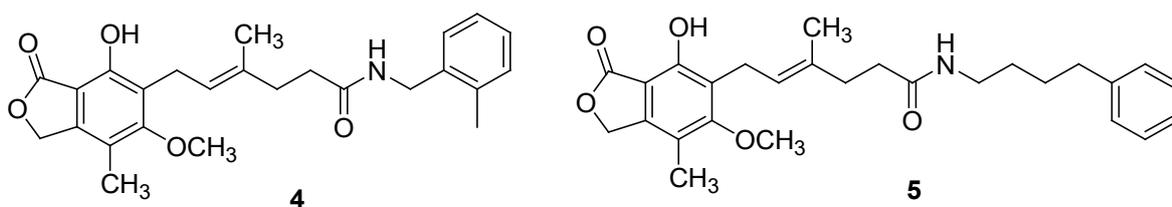
Fig. (2). Active *N*-alkyl amides of MPA **4, 5**.

Apart from anti-inflammatory and immunosuppressive properties, MPA derivatives were also considered as antiviral [25-28], antiparasitic [29,30], antifungal [31], and antimicrobial substance [29-33]. As a result, its inhibitory potency was not limited to human IMPDH (*Hs*IMPDH), but also *Mycobacterium tuberculosis* (*Mbt*IMPDH), *Staphylococcus aureus* (*Sa*IMPDH) and protozoan *Cryptosporidium parvum* (*Cp*IMPDH). Aniline derivatives with modified MPA side chains also showed activity variations due to structural differences between human (HsIMPDH2) and bacterial (CpIMPDH) enzymes [34]. In this series, 3-trifluoromethyl-4-chloro anilide **6** (Fig. **3**) revealed high potency Ki 0.016 μM *Cp*IMPDH, and Ki 0.23 μM *Hs*IMPDH2. Noteworthy, the reduction of alkene **6** to alkane **7** caused diminished activity to Ki 0.060 μM *Cp*IMPDH, however in the case of *Hs*IMPDH2, inhibition dropped much more and Ki was not determined. This

difference was rationally explained in terms of enzyme structures, where *Hs*IMPDH2 possesses hydrophilic serine at residue (Ser 276) interacting with the carboxylic group of MPA, which is properly situated in the NAD loop. On the other hand, *Cp*IMPDH contains in the equivalent position hydrophobic alanine (Ala 165).
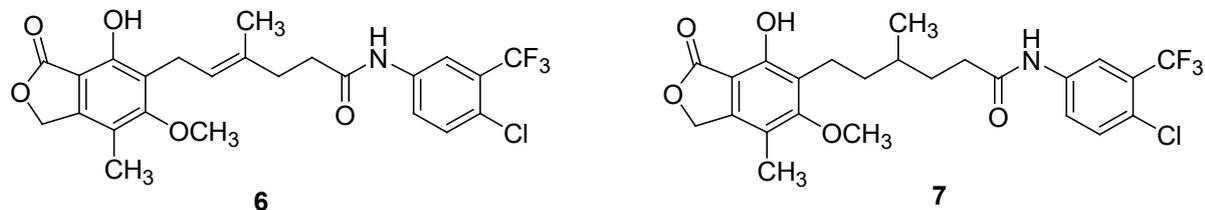
Fig. (3). MPA anilide **6** and its saturated counterpart **7**.

Inhibitory activities of other anilides bearing the modified side chain of MPA moiety were also correlated with structure differences between *Hs*IMPDH2 and prokaryotic types of enzymes. Unlike HsIMPDH2 where the NAD-binding region and active site reside within the same subunit, CpIMPDH has its active site located at the interface of adjacent subunits. Furthermore, tyrosine moiety (Tyr 358') of *Cp*IMPDH was proven to be important for its selective inhibition. Therefore, other activities were observed depending on the type of the enzyme. For instance, bioisosteric cyclopropane analogues **19**, **20** (Scheme **4**) of alkene **6** occurred to be more active against *Cp*IMPDH. These variations in conformation of the side chain modified alignment of MPA and aniline units. Moreover, the absolute configuration of cyclopropane had a greater effect on measured Ki of *Cp*IMPDH, where (1*R*,2*S*)-1-methylcyclopropyl diastereoisomer was more potent [34].

Synthesis of both cyclopropyl diastereoisomers started from MPA **1** (Scheme **1**). First, oxidation provided respective aldehyde, which was used in the Wittig reaction. Then, phenol was protected as *tert*-butyldimethylsilyl ether and the 2-methylbut-2-enal derivative underwent reduction to alcohol **8**.
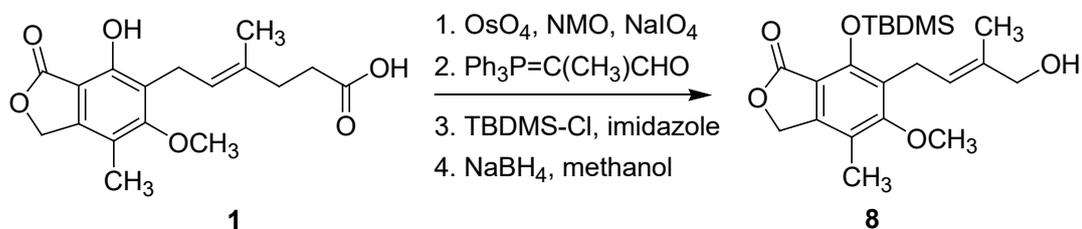
Scheme 1. Synthesis of intermediate **8**.

Subsequently, the methodology of Charette et al. [35] was used for chiral auxiliary preparation. Alcohol **8** reacted with 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-*α*-D-glucopyranosyl trichloroacetimidate **9** (Scheme **2**). Depending on Lewis acid, *β* **10** or *α* **11** anomer of D-glucopyranoside (Scheme **3**) was formed to enable further stereoselective cyclopropanations.
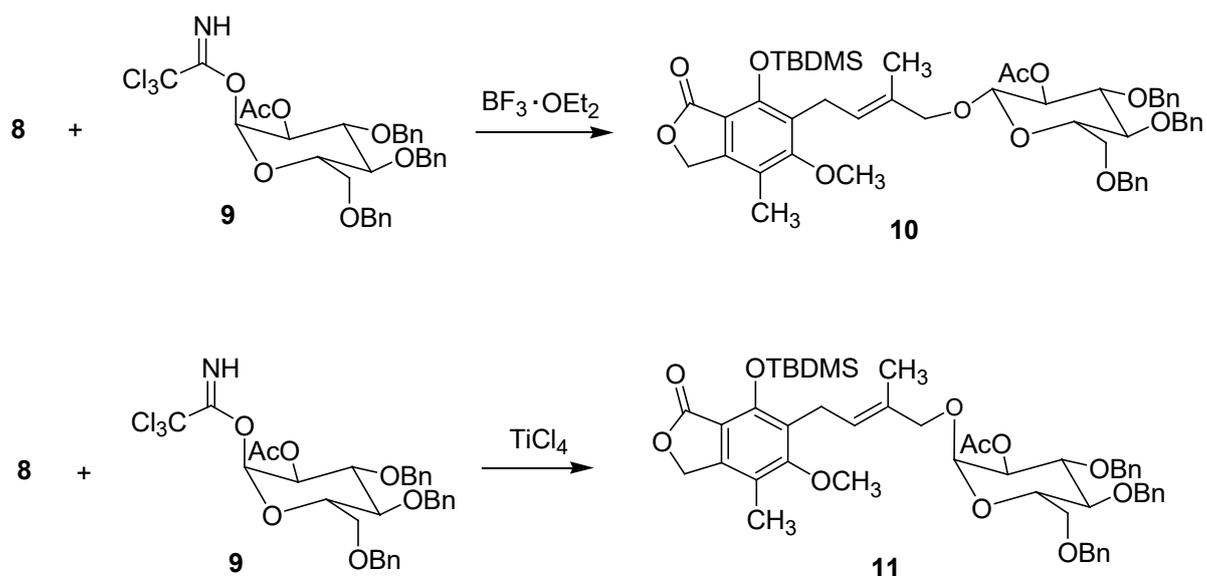
Scheme 2. Synthesis of anomeric intermediates **10** and **11**.

It was previously established that 2-hydroxyglucopyranosides provided improved diastereoselectivity of cyclopropanation of allylic ether in contrast to fully protected sugar units [35]. Therefore, **10** or **11** was treated with sodium methanolate to recover the 2-hydroxy group (together with phenol), and asymmetric cyclopropanation was performed with diethylzinc/diiodomethane (Scheme **3**) [34].
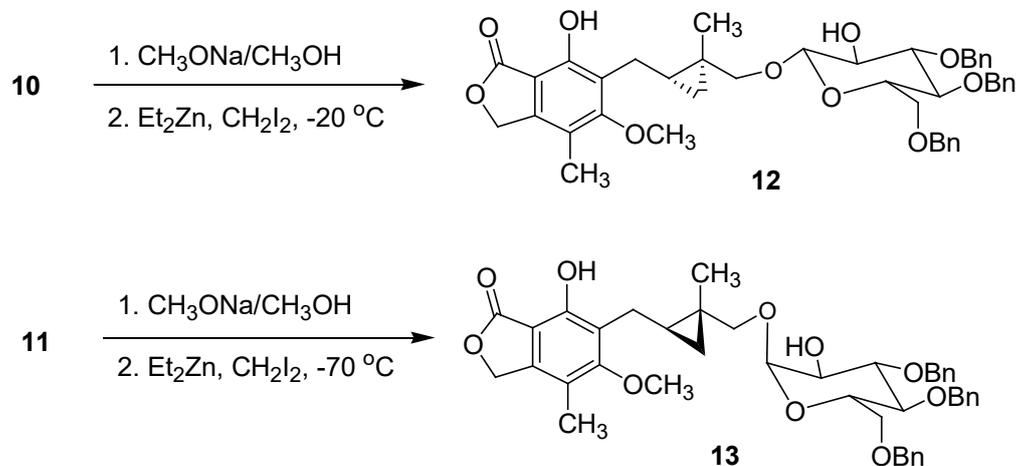
Scheme 3. Synthesis of cyclopropane intermediates **12**, **13**.

The non-racemic cyclopropylmethanol derivative **14** was liberated from its *O*-glycoside **12** in high yield followed by Dess-Martin periodinane (DMP) oxidation (Scheme **4**). Then, aldehyde **14** participated in the Horner-Wadsworth-Emmons reaction with triethyl phosphonoacetate **15** to ethyl acrylate derivative **16**, which underwent reduction to its saturated counterpart. Finally, ethyl ester was hydrolyzed with lithium hydroxide to cyclopropyl analogue of MPA **17**, which was coupled with substituted aniline **18** to produce target amide **19**. Diastereoisomeric compound **20** was obtained employing an analogical synthetic pathway from **13** [34].

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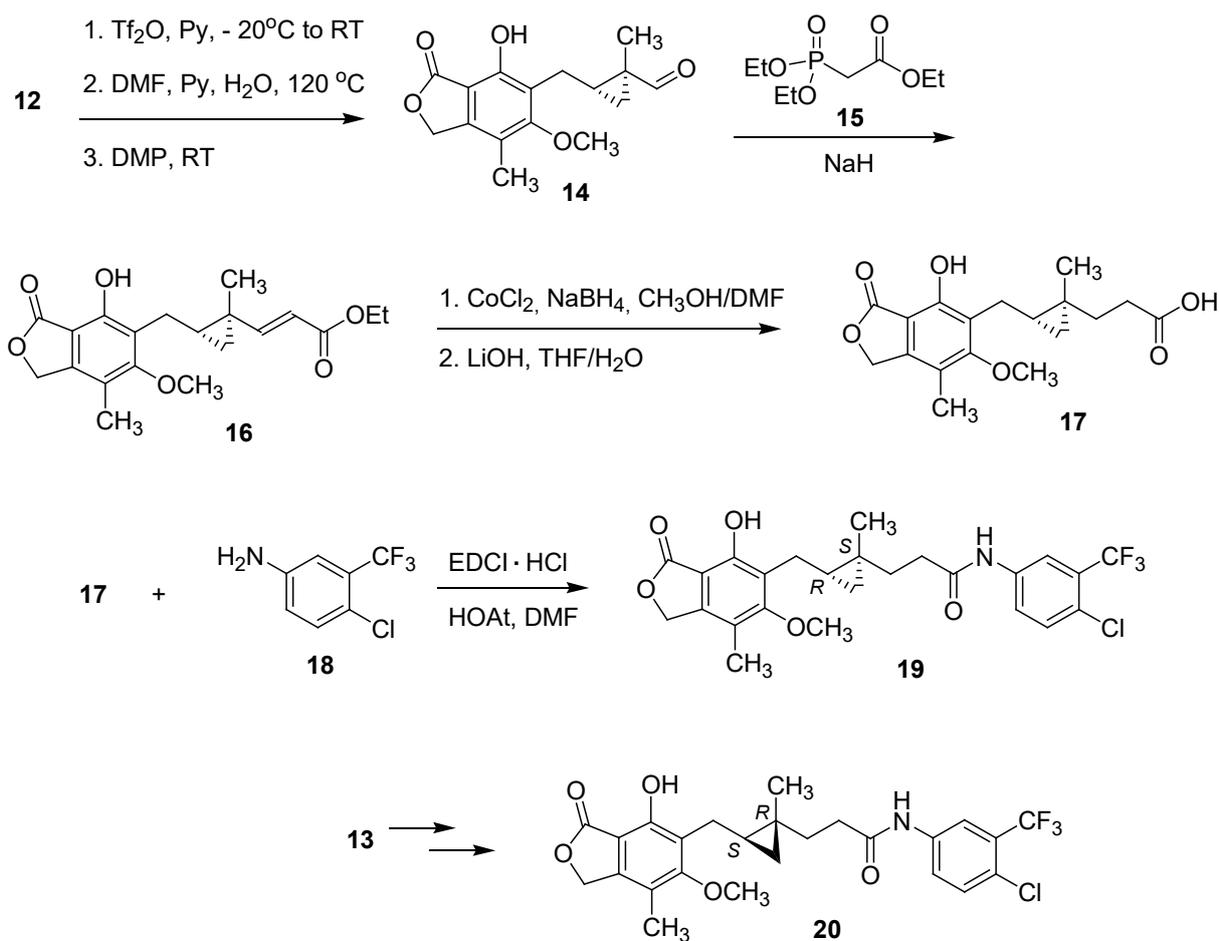
Scheme 4. Synthesis of cyclopropane analogues **19**, **20**.

Mycophenolic acid was also conjugated with amino acids and peptides to explore potential immunosuppressive, antibacterial, and anticancer properties [36-40]. For instance, designed conjugates of MPA **21** contained derivatives of tufsin, which is tetrapeptide H-Thr-Lys-Pro-Arg-OH possessing immunomodulating properties (Fig. **4**) [37], produced in the spleen. However, tripeptides derived from tuftsin lose their activity due to enzymatic breakdown. Therefore, structural analogs were investigated as potential immune stimulators. MPA was connected both with tuftsin and retro-tuftsin (reversed amino acid sequence) modified with an additional amino acid at the ε-amino group of lysine, like β-alanine or glycine.

R-	Asp(OCH$_3$)OCH$_3$	TyrLys(βAla)ProArg(NO$_2$)OCH$_3$
	ThrOCH$_3$	Arg(NO$_2$)ProLys(Gly)ThrOCH$_3$
	Arg(NO$_2$)OCH$_3$	Arg(NO$_2$)ProLys(βAla)ThrOCH$_3$
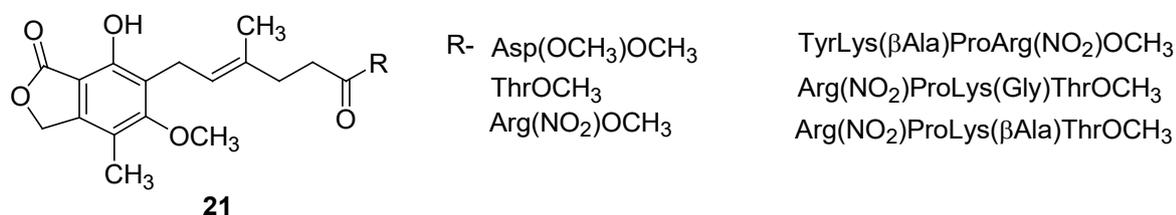
Fig. (4). Amino acid and tuftsin derivatives of MPA.

Similarly to the reaction of MPA with amino acid esters, coupling with suitably protected peptide derivatives was performed with EDCI/DMAP or T3P/NEt3 protocols [37,38].

Designed amino acid and tuftsin derivatives of MPA **21** revealed minimal inhibitory concentration (MIC) against methicillin-resistant *Staphylococcus aureus (*MRSA ATCC 43300) 32-64 µg/ml, which was lower

than parent MPA (750 μg/ml) and kanamycin (125 μg/ml) or ampicillin (128 μg/ml). Significant activity was also observed against *Klebsiella pneumoniae (*ATCC 700603) in the case of arginine and three investigated peptide derivatives. Noteworthy, natural L-configuration gave better microbiological activity than D-configuration in the amino acid moiety. Subsequently, hydrolysis of methyl esters to free carboxylic analogs provided decreased bioactivity. It could be explained by better cell membrane penetration caused by the lowered polarity of given compounds [39]. Secondly, these results were consistent with the structure of prokaryotic IMPDHs with no hydrophilic serine (Ser 276) in the NAD loop interacting with the free carboxylic group of MPA [34].

Both amino acid and tuftsin derivatives of MPA were also investigated *in vitro* against cancer cell lines in search of chemotherapeutics, which could be useful in the treatment of amelanotic melanoma and neuroblastoma. In this series, MPA-Thr-OCH$_3$, MPA-D-Thr-OCH$_3$, and MPA-Arg(NO$_2$)-OH amides occurred to be three times more active against hamster Ab melanoma cell line than dacarbazine, however, their activities against human melanoma cell line were weaker [40].

MPA **1** was also modified with various heterocyclic derivatives [41]. Recently were obtained amides bearing *N*-heterocyclic moieties *via* direct coupling of **1** with respective heteroaromatic amine as new IMPDH inhibitors. These condensation reactions proceeded without protection of phenol in MPA **1** including less nucleophilic amines possessing electron withdrawing substituents in heterocyclic ring. Although benzothiazole derivatives **22** (Fig. **5**) showed some drawbacks within poor solubility during *in vitro* investigations, their toxicity was low against human peripheral blood mononuclear cells (PBMCs), which might be advantageous in further development of their cytotoxic activity. The highest IC$_{50}$ 15.9 μM in this series of amides gave benzoxazole analogue **23**, which was similar to parent MPA **1** (10.5 μM) [42].

X: H, halogen, CF$_3$, NO$_2$, OCH$_3$
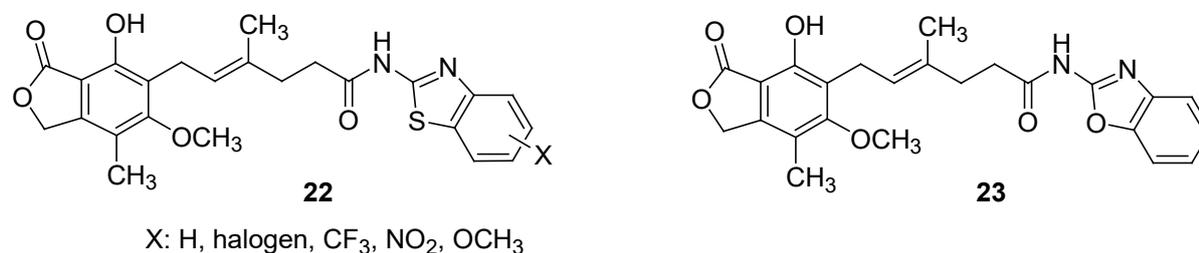
Fig. (5). MPA *N*-heteroaromatic amides with benzothiazole **22** and benzoxazole **23** moieties.

3. Corticosteroids

Corticosteroids act as agonists of glucocorticoid receptors (GR) and possess anti-inflammatory properties. These compounds are applied in clinics in the treatment of autoimmune diseases or immune disorders like asthma, chronic obstructive pulmonary disease, rheumatoid arthritis (RA), autoimmune hepatitis, systemic lupus erythematosus (SLE), and acute graft rejection [43-46].

Noteworthy, corticosteroids were also used clinically in coronavirus disease 2019 (COVID-19) treatment [47-50]. However, this usage may involve serious side effects, e.g. immunodeficiency against infections, hypertension, and hyperglycemia and there are explored structural modifications to improve the selectivity and effectiveness of the active substance [51].

The recommended nomenclature for the steroid skeleton of these compounds is based on sterane **24**, with marked fused rings and numbered carbon atoms of cyclopenta[*a*]perhydrophenanthrene moiety (Fig. **6**).

The known stereochemistry can be marked as absolute configuration *R, S,* and also as α (the considered bond lies below the plane of the paper), β (the considered bond lies above the plane of the paper) like in 5α-cholestane [52].
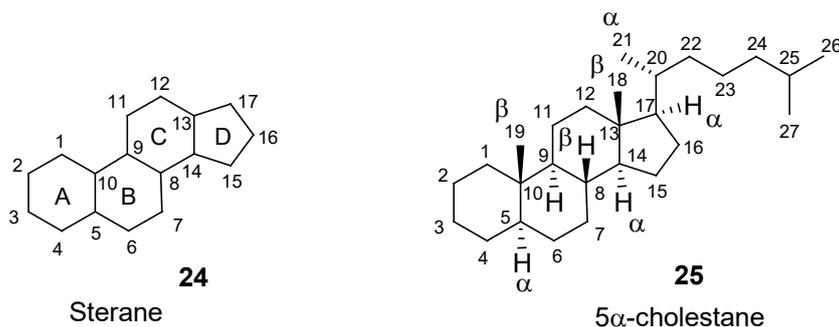
Fig. (6). Numbering of carbon atoms and designations of fused rings in steroid skeleton.

Hydrocortisone **26**, also known as cortisol, is a steroid hormone and an immunosuppressive drug used in medicine both in the form of injection or cream (Fig. **7**). This compound is also a substrate for steroids, with improved anti-inflammatory activity. Experimental protocols for obtaining this compound, including biosynthetic methods, were optimized. Furthermore, structural modifications were investigated towards increase of activity as GR agonist and improvement of therapeutic properties [53-60]. Similar properties are held by cortisone **27**, which is also a steroid hormone, and as a prodrug, it is metabolized to hydrocortisone **26** [61].
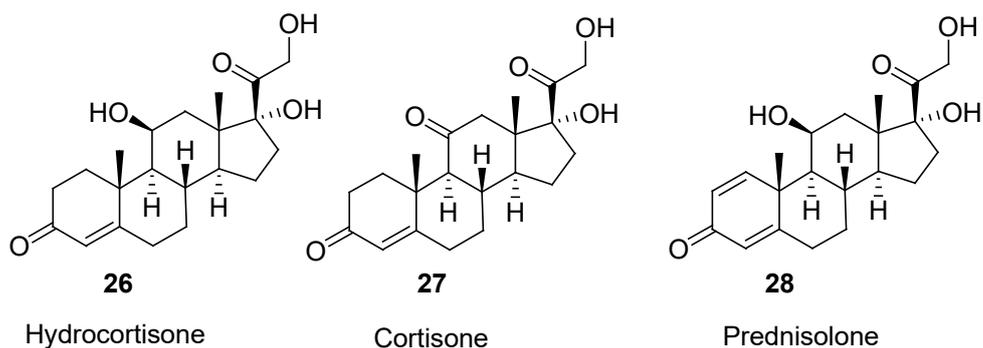
Fig. (7). Structures of hydrocortisone **26**, cortisone **27,** and synthetic derivative **28**.

Prednisolone **28** indicated 4-fold higher anti-inflammatory potency and differs from **26** in the presence of double bond at C-1. Although hydrocortisone **26** is less active than synthetically obtained prednisolone **28**, it is still widely applied in medicine [51,54,57,62,63].

Chemical synthesis of prednisolone **28** includes bromination of 21-acetoxy-11β,17α-dihydroxy-5α-pregnan-3,20-dione **29** followed by elimination of hydrogen bromide with 3,5-dimethylpyridine and subsequent hydrolysis of respective acetate (Scheme **5**). Analogically, dihydrocortisone acetate **30** can be converted to prednisone **31**. Both steroids possessing carbonyl group at the C-11 position are used as anti-inflammatory drugs in autoimmune disorders treatment [54,64].
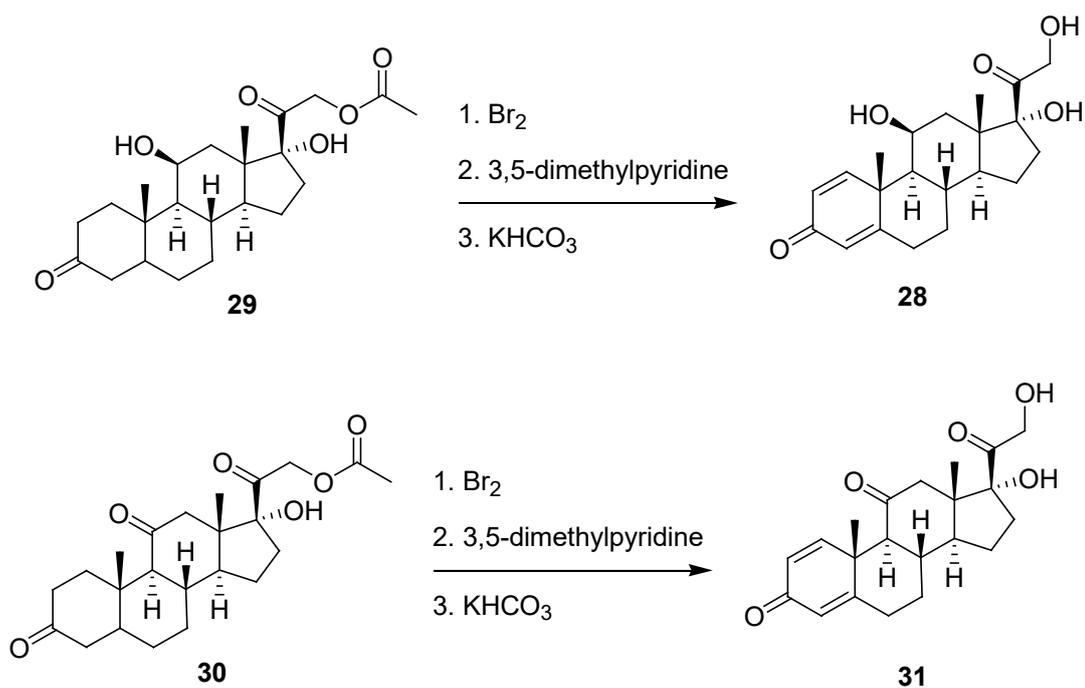
Scheme 5. Synthesis of prednisolone **28** and prednisone **31**.

However, these chemical protocols possess several disadvantages concerning region- and stereospecificity, and some biotransformation were developed to produce prednisolone and its derivatives under mild conditions and aqueous media. Recently were reported Δ1-dehydrogenations of hydrocortisone **26** and cortisone **27** by *Rodoccocus strains* in high yields to prednisolone **28** and prednisone **31**, respectively [54].

Modification of prednisolone **28** in terms of the addition of methyl group at C-6 improves slightly anti-inflammatory properties. 6α-Methylprednisolone **32** (Scheme **6**) is used in medicine as an immunosuppressive agent to treat e.g. rheumatic disorders, hematologic and dermatologic problems, respiratory and gastrointestinal diseases [51,55].
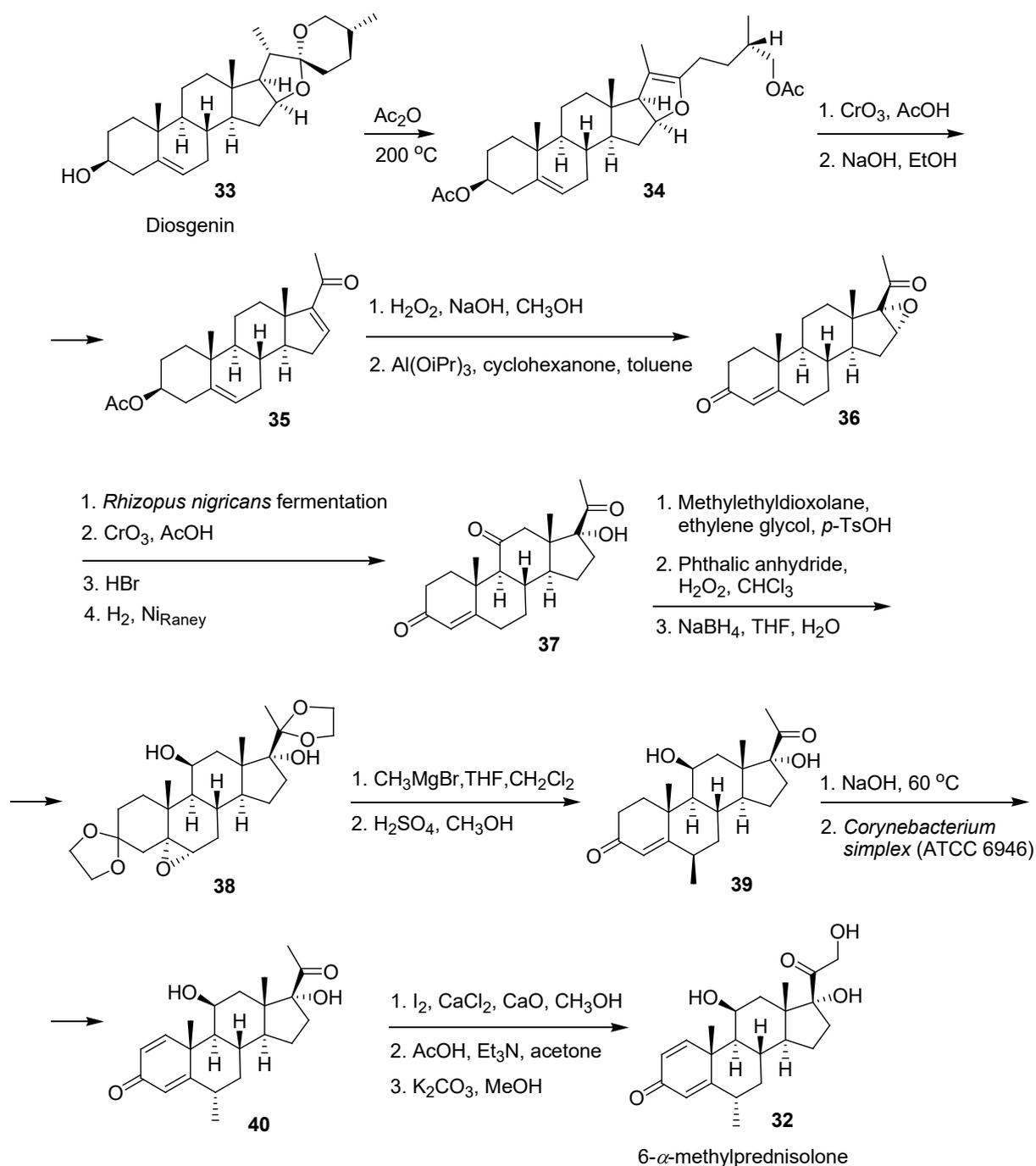
Scheme 6. Synthesis of 6α-methylprednisolone **32**.

6α-Methylprednisolone **32** can be synthesized from *Diosgenin* **33**, naturally occurring sapogenin (Scheme **6**). First, treatment with acetic anhydride under heating provides acetate **34** through the elimination and opening of cyclic acetal moiety. Then, oxidation of the double bond with CrO_3 gives, apart from ketone, ester, which upon hydrolysis and elimination leads to α,β-unsaturated ketone **35**. Oxidation with H_2O_2 and in basic conditions produced epoxide bearing secondary hydroxyl group at C-3, followed by its oxidation using the Oppenauer method together with isomerisation of alkene to α,β-unsaturated ketone **36**. Subsequently, the fermentation technique was applied to (*R*)-hydroxylation at the C-11 position, and respective alcohol was

oxidized to ketone with CrO_3. Next, epoxide was cleaved with HBr to relevant α-halogenoalcohol, where bromide was removed utilizing catalytic hydrogenation to **37** [55].

Selective conversion of the two ketone groups to cyclic acetals occurred with the alkene C-4 to C-5 isomerisation, which was, in turn, oxidized to 5,6-epoxide upon H_2O_2 treatment, and then, ketone C-11 reduced with NaBH4 to (*S*)-alcohol **38** stereospecifically (Scheme **6**). Subsequently, the epoxide system in **38** was cleaved with Grignard reagent to (*R*)-methylated (*β*-methylated) deriver at the C-6 position, and alcohol was dehydrated to C-4 alkene **39**. Diastereoisomer **39** underwent conversion to *α*-methyl at C-6 by heating with NaOH with a high diastereomeric excess of 94% followed by oxidation to C-2 alkene **40** using *Corynebacterium simplex* (ATCC 6946). Finally, the primary hydroxyl group was introduced with the Ringold-Stork protocol, *via* iodination, subsequent acetate formation, and then ester hydrolysis in basic conditions to produce primary alcohol **32** [55].

3.1. Fluorinated corticosteroids

Fludrocortisone **41** (9*α*-fluorocortisol or 9*α*-fluorohydrocortisone) was the first drug containing and also the first marketed synthetic corticosteroid (Scheme **7**) [58] with high mineralocorticoid activity (electrolyte regulation), often accompanied by weak glucocorticoid activity (antiinflammatory as GR agonist). Due to its solubility in lipids, it can penetrate cell membranes and bind to its cytoplasmic receptor, forming a complex that translocate to the nucleus. Fludrocortisone primarily acts in the distal tubules and collecting ducts of the kidneys, promoting sodium and water reabsorption while increasing potassium and hydrogen ion excretion [59]. Its properties allow it to be used as acetate in hypotension, hypovolemia, Addison's disease, and adrenal cortex treatment [60]. Fludrocortisone **41** can be prepared from hydrocortisone acetate **42**, which undergoes elimination with phosphoryl chloride in pyridine to C-9 alkene followed by respective bromohydrin formation upon *N*-bromoacetamide addition. Subsequently, sodium acetate gave epoxide **43**, which was cleaved with HF to produce fludrocortisone acetate **44**. Acetate can be removed with alkaline hydrolysis to **41** [57].

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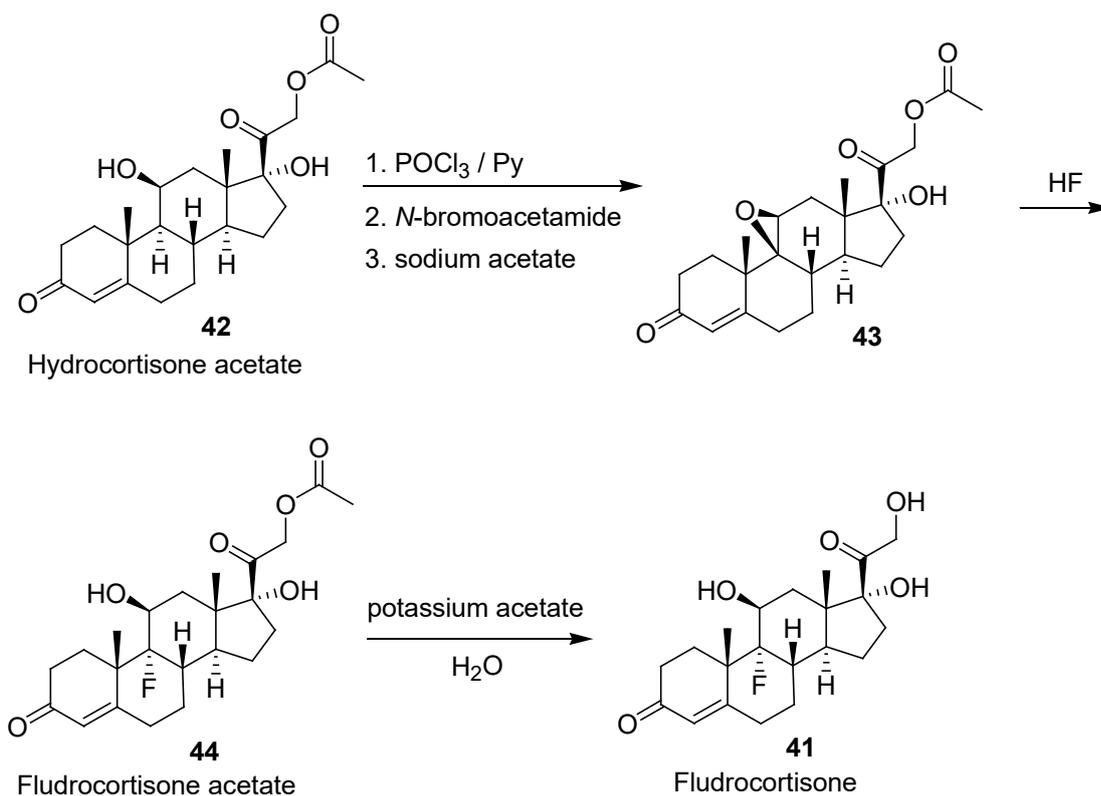
Scheme 7. Synthesis of fludrocortisone **41**.

Dexamethasone **45** and betamethasone **46** (Fig. **8**) are characterized with 5-fold higher anti-inflammatory activity in comparison to 6α-methylprednisolone **32** [51]. They possess fluoride at the C-9 position and methyl substituent at C-16, where dexamethasone **45** is an α-analog. These fluorinated corticosteroids are used for the treatment of women at risk of pattern delivery to minimize neonatal death, respiratory distress syndrome, or disorders including other organ maturation. Both dexamethasone **45** and betamethasone **46** were recommended, however less expensive dexamethasone **45** possesses shorter half-time, and it can be better within the long-term effect for diabetic mothers. On the other hand, betamethasone **46** was more effective in the prevention of respiratory complications after cesarean section [65-67].
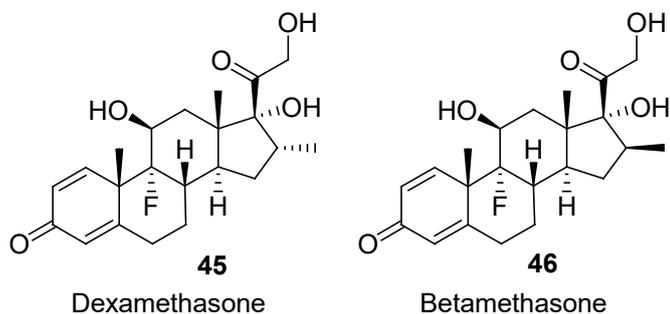
Fig. (8). Structures of dexamethasone **45** and its diastereoisomer betamethasone **46**.

Synthesis of both compounds are multistage conversions and apply chemical and biotechnological transformations. First, starting compound **36** underwent two-stage biotransformation including Δ1-dehydrogenation followed by hydroxyl group at C-11 introduction to **47** (Scheme **8**). Then, both hydroxyl and epoxide were converted to alkene moieties at C-9 and C-16 of **48**, respectively. Subsequently, the addition of *N*-

bromosuccinimide to C-9 alkene and oxidation with perchloric acid gave respective bromoalcohol, which cyclize to 9,11-epoxide **49**. Double bond C-16 underwent the addition of methylmagnesium chloride with oxidation to 16-α-methyl-17-α-hydroxyl derivative, followed by a reaction of 9,11-epoxide with hydrogen fluoride to **50**. Finally, the iodination of methyl ketone allowed the introduction of respective acetate, which was hydrolyzed to dexamethasone **45** [55].
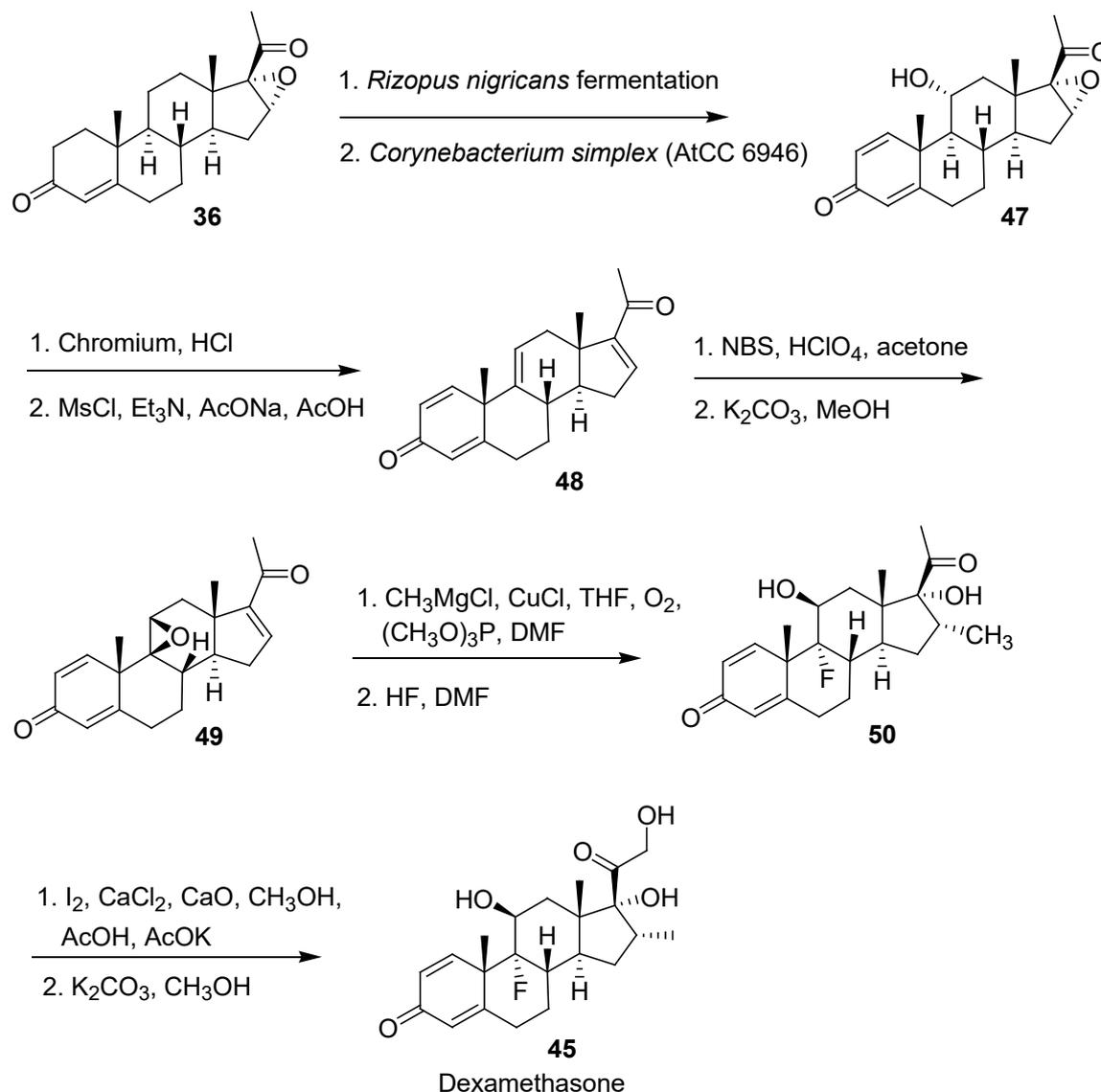
Scheme 8. Synthesis of dexamethasone **45**.

Betamethasone **46** is a 16-*β*-methyl stereoisomer of dexamethasone **45** and this stereochemistry was achieved in several routes [55,68,69].

Methyl ketone moiety in intermediate **51** was brominated and substituted with acetate followed by oxidation of C-3 secondary alcohol to ketone (Scheme **9**). Subsequently, the obtained ketone was *α*-dibrominated and eliminated to the respective diene. *S*-hydroxylation at C11 was performed with enzymatic procedure together with acetate hydrolysis to **52**. Primary alcohol **52** was protected in reaction with ethyl chloroformate, and C-11 hydroxyl was eliminated using PCl5, while the resulting alkene was converted with 1,3-dibromo-5,5-

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dimethylhydantoin (DBH)/HClO4 to bromoformate **53**. Subsequently, both carbonate and formate species were hydrolyzed, whereas produced bromohydrin gave respective 9,11-epoxide, which underwent cleavage using HF to betamethasone **46** [55].
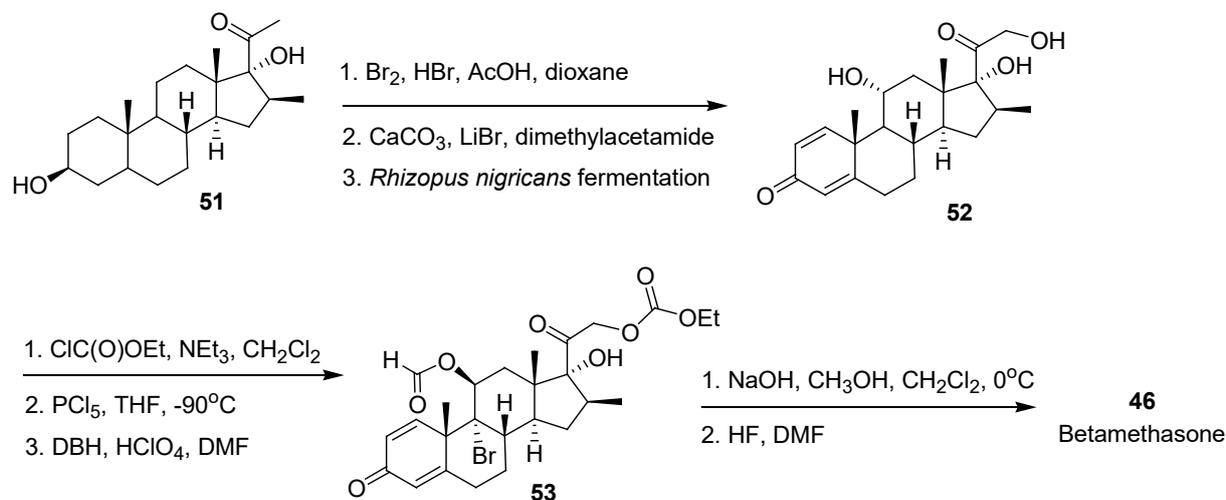
Scheme 9. Synthesis of betamethasone **46**.

In the search for interesting biological properties, modifications including fluorine introduction into steroid skeleton were intensively studied. One of the important stages in the synthesis of dexamethasone or betamethasone was epoxide cleavage with HF to the respective 9α-fluoroderivative. Furthermore, 6α,9α-difluoroderivative **54** (Scheme **10**) is a useful intermediate in the synthesis of fluticasone **55** and its derivatives **55,56** (Fig. **8**) possessing significant anti-inflammatory properties. First, 5α, 6α-epoxide **58** cleavage led to 6β-fluorine analog **59**, where electronic and steric aspects were discussed. The inversion of the configuration of **60** into 6α-fluoride **61** was performed upon enolization with HCl — an acetic acid system [69,70]. Subsequently, enzymatic oxidation of **61** gave 16-β-hydroxy derivative **62**, which underwent elimination *via* mesylate. The resulting C-9 alkene was converted, with generated *in situ* hypobromous acid, into 9β,11β-epoxide **63**, followed by cleavage to 6α,9α-difluorocompound. Finally, oxidation with selenium dioxide produced 6α,9α-difluro-16α-methylprednisolone 21-acetate **54** [70,71].
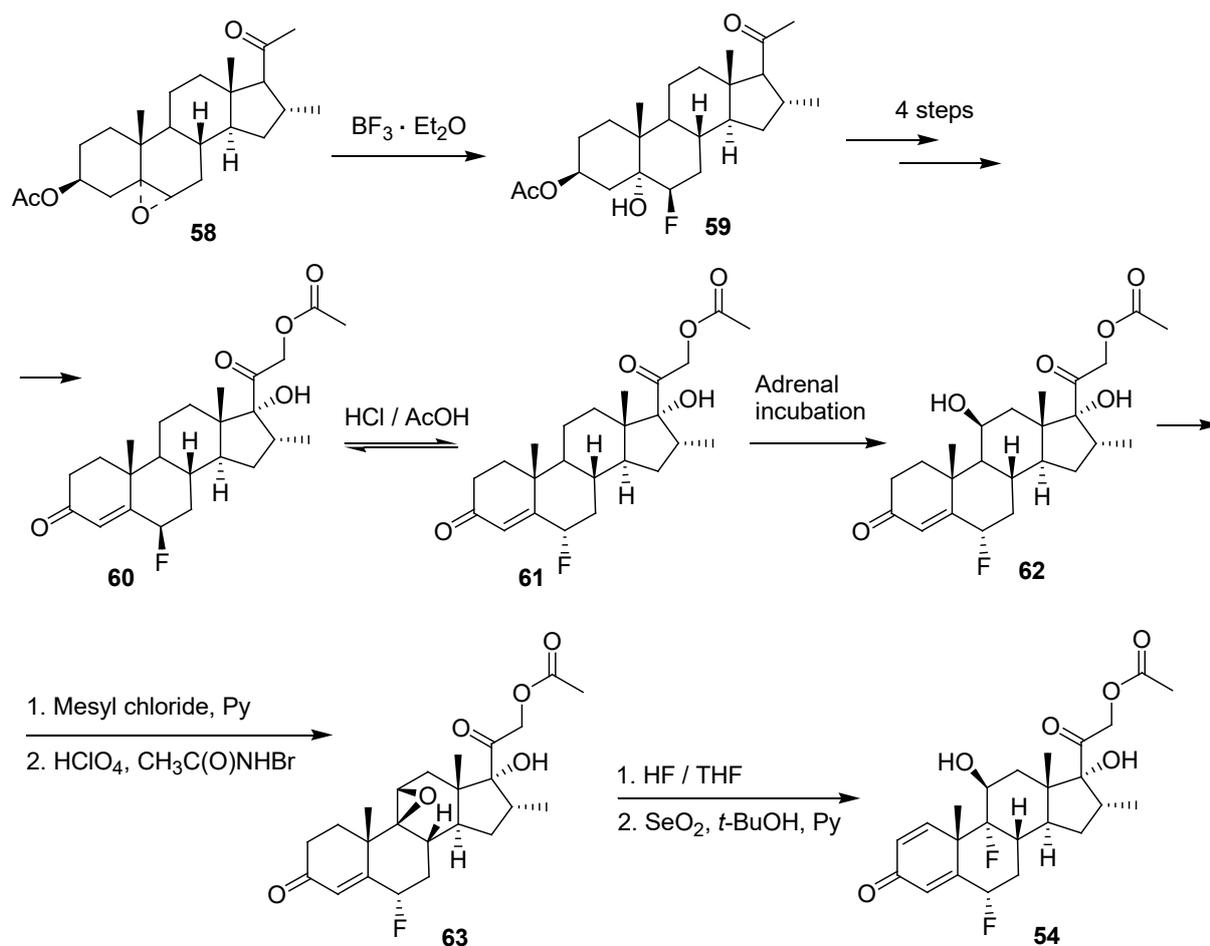
Scheme 10. Synthesis of an intermediate **54**.

The steroid derivative **54** is one of the possible intermediates in the synthesis of fluticasone **55** and its esters **56,57** (Fig. **9**), which are important inhaled glucocorticoids applied widely in the treatment of asthma and allergic rhinitis [72-76].
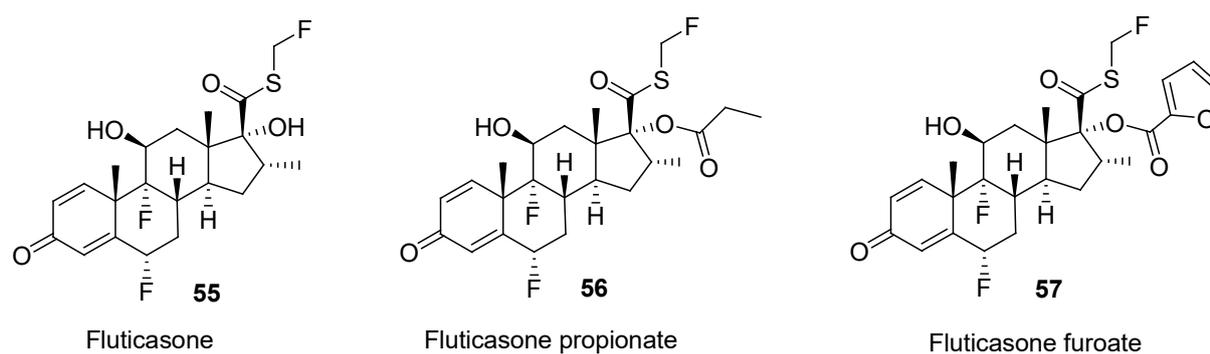
Fig. (9). Structure of fluticasone **55** and its clinically applied esters **56,57**.

In the synthetic pathway of fluticasone propionate **56** (Scheme **11**), C-21-acetate **54** was hydrolyzed, and hydroxymethyl ketone oxidized with orthoperiodic acid to 17β-carboxylic acid **64**. Treatment of this compound with an excess of acylating agent gave both propionyl at 17β-carboxylic and 17α-hydroxyl moieties. Subsequently, selective aminolysis produced 17α-propionyloxy-17β-carboxylic acid **65**. The carboxylic group was converted to 17β-carbothiate *via* activation with carbonyldiimidazole (CDI) in the substitution at acyl

carbon atom with sodium hydrosulfide. Then, the reaction with bromofluoromethane was one of the possible methods to form *S*-fluoromethyl ester **56**.
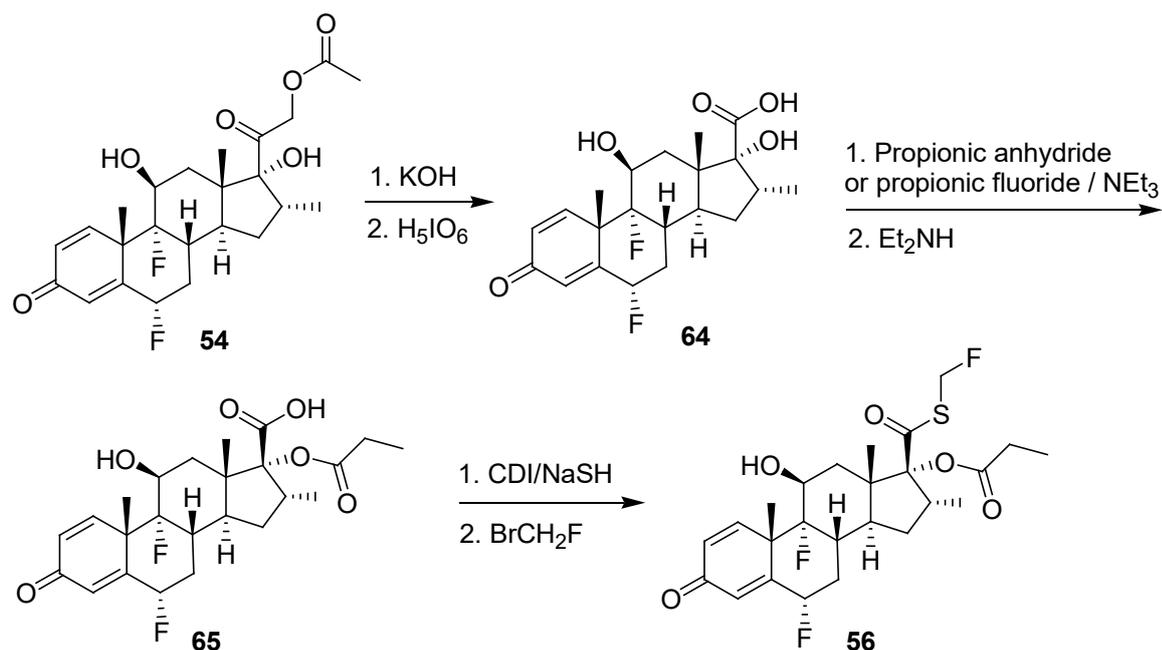
Scheme 11. Synthesis of fluticasone propionate **56**.

Zhou and co-workers reported an improved synthetic route towards fluticasone propionate **56** (Scheme **12**) to avoid expensive and hazardous reagents like H5IO6 or monofluoromethylation reagents [75].
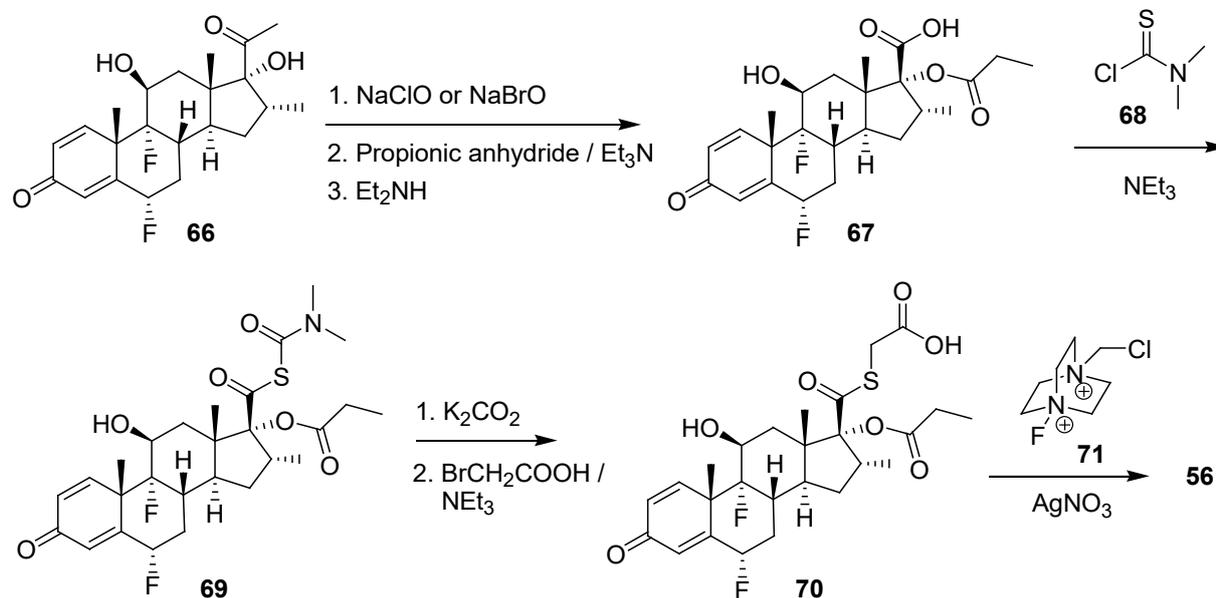
Scheme 12. Synthesis of fluticasone propionate **56** from commercially available intermediate **66**.

Commercially available 17*β*-methylketone **66** was oxidized with sodium hypochlorite or sodium hypobromite to 17*β*-carboxylate, followed by acylation to isolated 17*α*-propionyloxy-17*β*-carboxylic acid **67**. Then, the reaction with *N,N*-dimethylthiocarbamoyl chloride **68** gave upon nucleophilic substitution and apparent isomerisation *S*-diacylated intermediate **69**, which underwent hydrolysis to respective 17*β*-carbothiate.

Subsequently, this thiocarboxylic moiety was converted with bromoacetic acid to *S*-acylated sulfanylacetic acid derivative **70** and its decarboxylation with selectfluor **71** produced fluticasone propionate **56**.

Acyl counterpart of fluticasone propionate — a furoate analog **57** can be prepared in a similar way (Scheme **13**) [74]. Selective acylation of C-17 tertiary hydroxyl **72** was carefully optimized. Thiocarboxylate underwent predominantly *S*-acylation with 2-furoyl chloride and preferred intramolecular acyl transfer led to the respective 17-*α*-2-furonyloxy derivative. An excess of *S*-2-furoyl mixed anhydride was decomposed with diethyl amine and recovered 17*β*-carbothiate reacted with bromofluoromethane to give fluticasone furoate **57**.
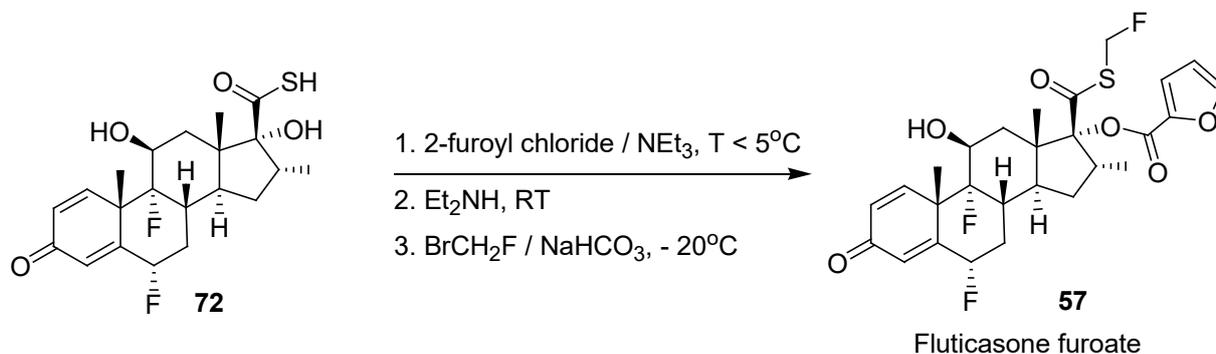
Scheme 13. Synthesis of fluticasone furoate **57**.

3.2. Budesonide and its derivatives

The next example of a potent inhaled corticosteroid used against asthma or chronic obstructive pulmonary disease is budesonide **73** (Scheme **14**). Chemically, it is acetal derived from 16-*α*-hydroxyprednisolone **74** and butyraldehyde, where the two stereoisomers at the acetal carbon atom (C-22) are obtained [77-79]. Starting 16-*α*-hydroxyprednisolone **73** can be prepared e.g. enzymatically from hydrocortisone **26** [80].
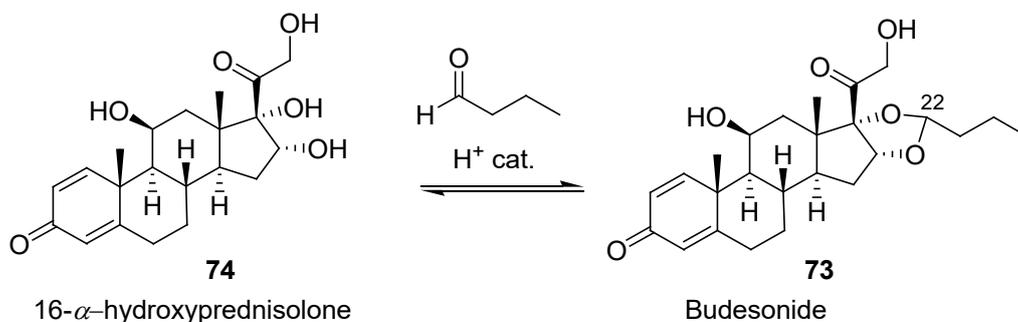
Scheme 14. Direct synthesis of budesonide **73**.

Since corticosteroids can cause significant adverse effects concerning diabetes, hypertension, myopathies, and osteoporosis, Ghidini and co-workers designed novel pyrrolidine derivatives of budesonide **75** (Fig. **10**) [81]. The most promising anti-inflammatory properties revealed *m*-chlorobenzyl analog **76** including higher binding activity towards glucocorticoid receptor (Ki 0.76 nM) than budesonide **73** (Ki 1.3 nM) together with lower NO release and improved both lung retention and neutrophilia in a rat model.
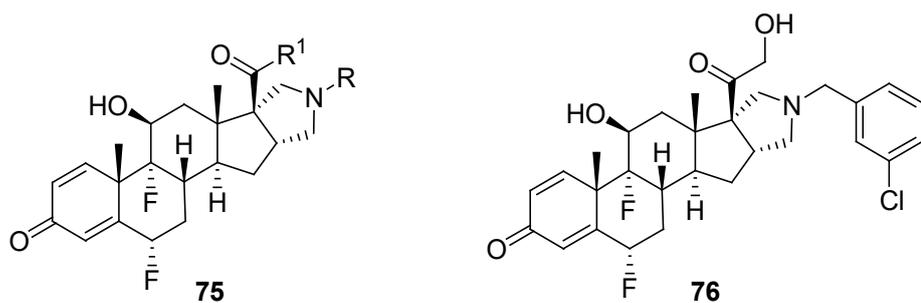
Fig. (10). Budesonide derivatives **75, 76**.

The starting material, commercially available butyric ester **77** (Scheme **15**), was subjected to an elimination reaction. Then, corresponding alkene **78** underwent cycloaddition with *N*-benzyl-1-methoxy-*N*-((trimethylsilyl)methyl)methanamine **79** to form a pyrrolidine moiety. Subsequently, tertiary amine **80** was debenzylated with vinyl chloroformate followed by the decomposition of respective vinyl carbamate to adequate secondary amine hydrochloride **81**. Then, *N*-alkylation of amine and acetate hydrolysis gave target compound **76** [81].
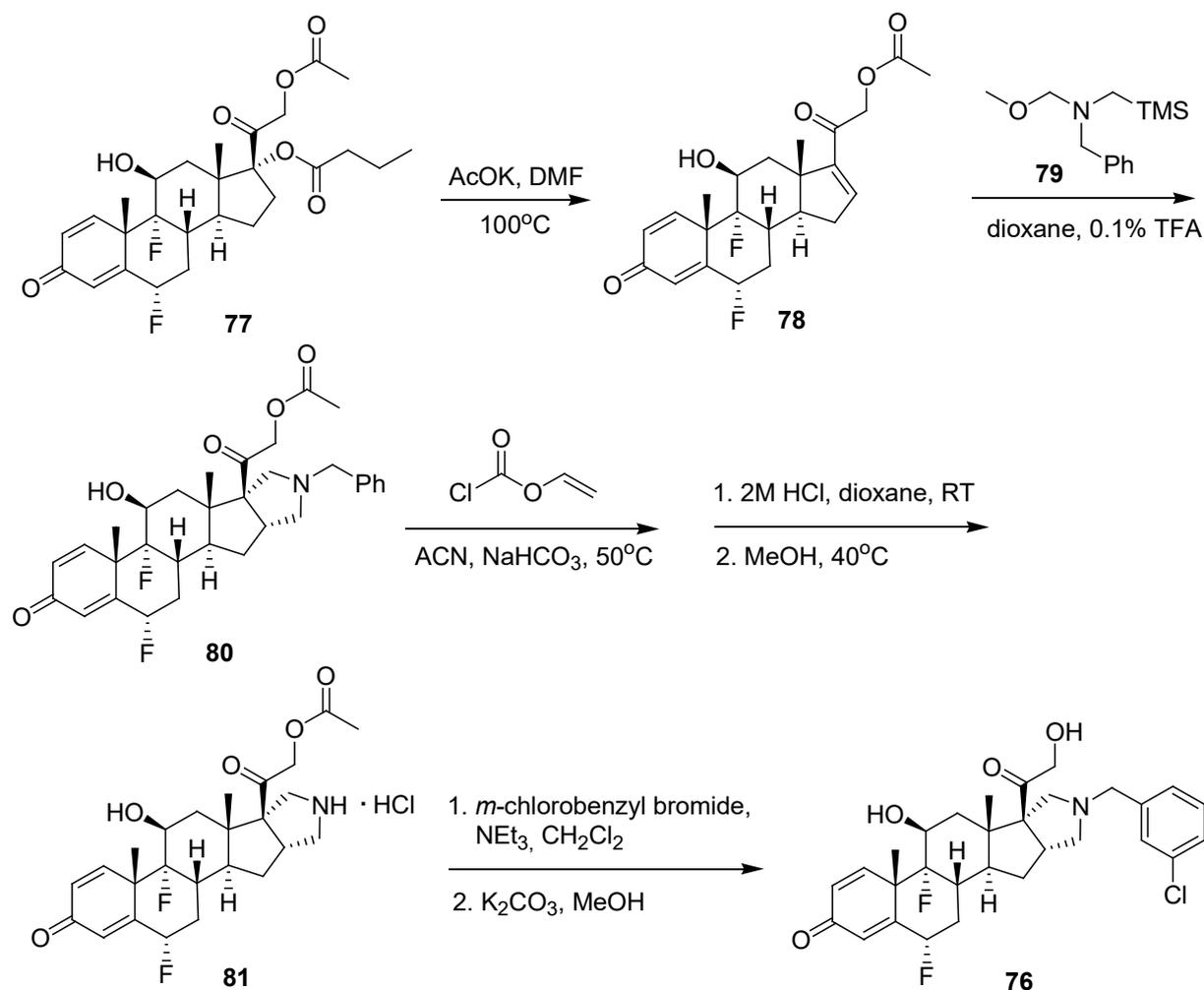
Scheme 15. Synthesis of budesonide derivative **76**.

The poor water solubility of budesonide **73** is a significant factor influencing its anti-inflammatory potency. Recently, amino acid conjugates were designed and characterized to improve their dissolution [82].

Thus, budesonide **73** was coupled with Boc-protected glycine, phenylalanine, or *β*-alanine followed by deprotection upon trifluoroacetic acid treatment to respective esters **82**, **83** (Scheme **16**). The second series of conjugates **84** was formed in the reaction of budesonide **73** with chloroacetyl chloride. Subsequently, nucleophilic substitution in the presence of sodium iodide as a catalyst gave diethylamine, dimethylamine, *N*-methylpiperazine, and morpholine derivatives, respectively. The best solubility properties revealed glycine (180-fold), *β*-alanine (149-fold) and dimethylamine (16-fold) counterparts in comparison to **73** glycine and *β*-alanine analogs were the most stable against hydrolysis together with IL-6 on LPS - induced A549 cell line inhibition and promising anti-inflammatory activity *in vivo* in xylene-induced ear edema test in mice.
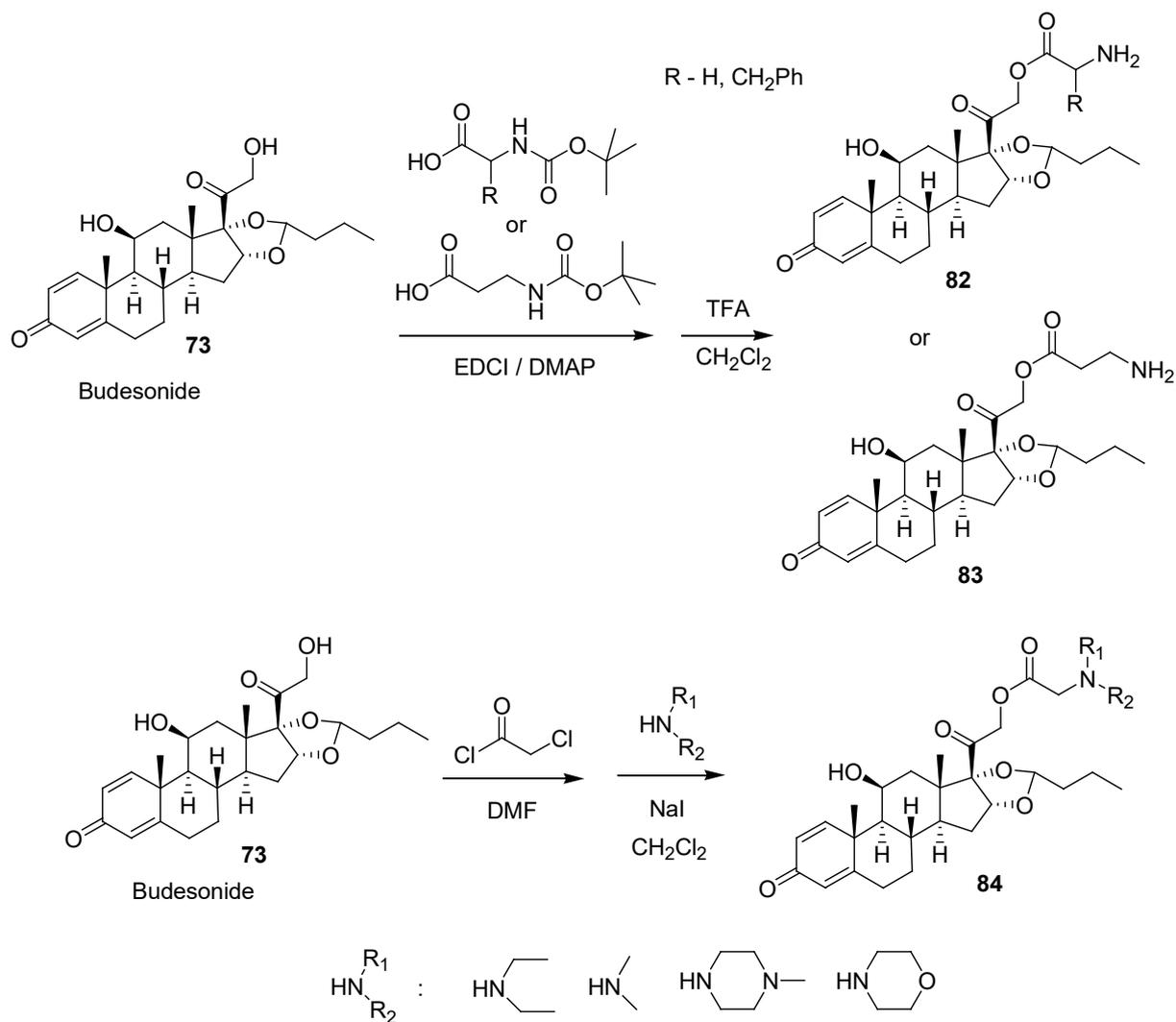
Scheme 16. Synthesis of amino acid conjugates of budesonide **82-84**.

4. Heterocyclic-based immunosuppressants

Heterocyclic compounds offer multiple classification options due to the wide range of physicochemical and biological characteristics that can be observed within this category of organic substances. To organize the given ones, one may divide them according to the number and type of heteroatoms, coherent bioactivities, and the size of the ring. The latter approach is used beneath with the differentiation of 5-membered, 6-membered, and fused rings.

Due to the high structural constraints, 3-membered systems are rather used as alkylators for carcinogenesis-related purposes or the fight against microbial infections [83-85]. One's attention needs to be drawn to less rigid, 4-membered rings which tend to present a notable correlation between molecular rigidity and satisfactory stability. In turn, they may be thought to present a wider range of bioactivities than their 3-membered counterparts [85,86]. Nevertheless, their microbial-oriented activities deserve special attention since 12 out of 17 antimicrobial fused ring-based compounds turned out to have the oxetane structural motif incorporated within their structures [87].

The basic concept standing behind this study is to depict examples in which the discussed heterocyclic system is separated from the rest of the molecule by at least one single bond. Additionally, one may become acquainted with the lapidary pre-description of each of the compounds in a form of a theoretical introduction presenting the mechanism of action of the drug/potential drug and, if applicable, a short description of the aetiology of the disease it concerns, in the clearest possible way.

The set of compounds with immunosuppressive properties presented herein shows several mechanisms of action related to specific diseases. Among them, one may enumerate inhibitory activity towards IMPDH, Janus-activated kinases (JAK), Bruton's tyrosine kinases (BTK), spleen tyrosine kinase (SYK), serum and glucocorticoid-regulated kinase 1 (SGK1) or dihydrofolate reductase (DHFR), as well as more distinguished inhibitory properties of such systems: p22phox (NADPH oxidase complex subdomain, NOX), perforin, the translocator protein (TSPO) or programmed cell death protein 1/programmed cell death ligand (PD-1/PD-L1). The latter system presents rather immunomodulating properties due to anti-antiproliferative activity, however, with the important immunotherapeutic strategy indication. Additionally, another molecular mechanism such as the sphingosine-1-phosphate (S1P1) receptor internalisation was depicted. Collectively, described compounds have or may have an application in conditions such as prophylaxis of graft rejection, inflammation, myeloproliferative neoplasms (MPNs), RA, SLE, multiple sclerosis (MS), systemic sclerosis (SSc), lupus nephritis (LN) or chronic lymphocytic leukaemia (CLL) and small lymphocytic lymphoma (SLL).

4.1. 5-membered rings

4.1.1. Imidazole (Mizoribine)

Mizoribine (**92**, also known as bredinin) is an immunosuppressive drug classified as an imidazole nucleoside agent. It offers a feasible alternative to azathioprine (AZA) in immunosuppression, as it has been found to have significantly lower toxicity levels. It has shown promising results as a safe and effective immunosuppressant in human kidney transplantation. Due to its antiviral properties, mizoribine could also be a possible candidate for evaluation in the treatment of BKVN (BK virus nephropathy) [88,89].

The immunosuppressive effects of mizoribine are achieved through the selective inhibition of two enzymes: IMPDH and guanosine-5'-monophosphate synthetase (GMPS) which are essential for the production of guanosine-5'-monophosphate (GMP) from inosine-5'-monophosphate (IMP) in the *de novo* pathway. Mizoribine has been found to inhibit both humoral and cellular immunity by selectively suppressing lymphocyte proliferation [89].

Mizoribine is prescribed for renal transplantation due to its immunosuppressive properties, which are similar to those of AZA. However, unlike AZA, mizoribine does not become incorporated into nucleic acids within cells. This property reduces the risk of adverse effects such as myelosuppression and hepatotoxicity.

Mizoribine also finds application in the management of various renal conditions. It is particularly useful for patients with nephrotic syndrome, especially the steroid-resistant kind, who do not respond well to steroid treatment; for paediatric patients with lupus nephritis (LN) who are dependent on steroids for symptom control, where high-dose mizoribine therapy has demonstrated effective results and provides adequate protection, justifying its use [89].

One of the synthetic approaches, presented in Scheme **17**, towards this compound assumes the use of acyclic substrates, simple (and cheaper than in the case of cyclic substrates), which in the further stages of this transformation were cyclized to the imidazole ring.
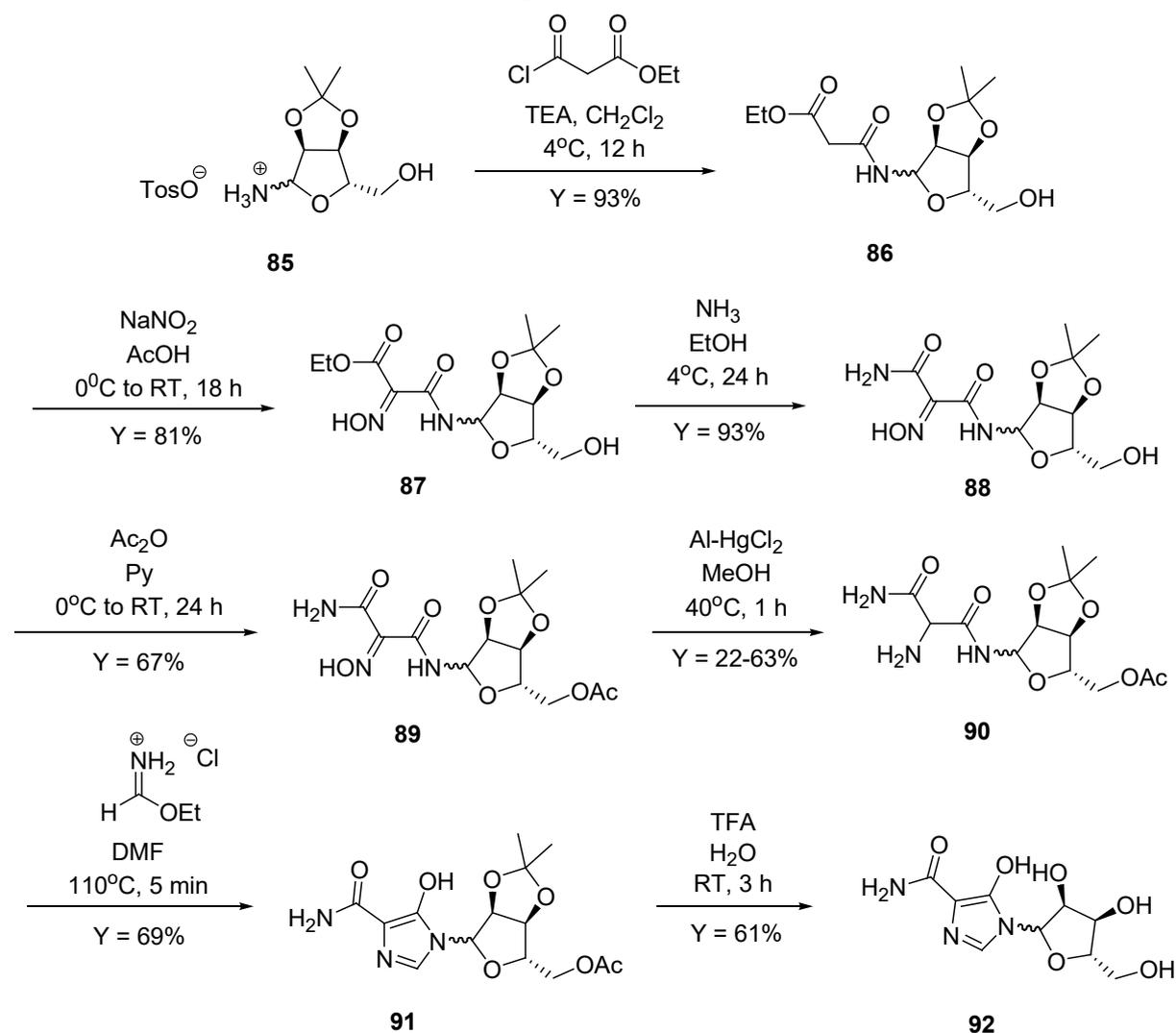
Scheme 17. Synthetic approach towards Mizoribine (**92**).

The first step is represented by *N*-acylation of **85** with ethyl malonate chloride in the presence of TEA. Isonitrosomalonate-type compound, **87**, was obtained by treating **86** with sodium nitrite in acetic acid. Then, by using an ethanolic ammonia solution, one may attain amide **88** *via* ester aminolysis. Acetylation of the sugar hydroxyl group was performed using acetic anhydride in the presence of pyridine, giving **89**. The isonitrosomalonate part of the compound was successfully reduced with Al-HgCl$_2$ amalgam, thus generating **90**. Depending on the reaction scale, one may acquire different percent yields of the product. Cyclization was carried out using ethyl formimidate hydrochloride under increased temperature. The obtained **91** intermediate was then

treated with trifluoroacetic acid to remove isopropylidene and acetyl protective groups from sugar hydroxyl moieties attaining target molecule, **92**. The product exists as a mixture of anomers. It is difficult to isolate just one of them [90]. It is possible to obtain only one anomer using another method, i.e. transformation of inosine, with full preservation of the desired stereochemistry [91].

4.1.2. Pyrazole (Ruxolitinib)

Ruxolitinib (**100**), also known as Jakafi, is classified as a Janus-activated kinases inhibitor and is primarily used to treat myelofibrosis and polycythemia vera, i.e. myeloproliferative neoplasms (MPNs), which are a group of rare blood disorders characterized by the abnormal production and function of blood cells in the bone marrow. The main mechanism of action of ruxolitinib is to inhibit the phosphorylation of signal transducer and activator of transcription (STAT) proteins by JAK. This inhibition affects cell divisions as well as the induction of programmed cell death [92,93]. It is a selective inhibitor of the ATP-binding site of JAK1, which plays a role in regulating interleukin 2 (IL-2), interleukin 6 (IL-6), and tumour necrosis factor-alpha (TNF-α), as well as a JAK2, which is involved in various cellular functions, including cell proliferation and differentiation.

(*R*)-Ruxolitinib (**100**) may be obtained through multi-step transformation which comprises: copper(I)-catalyzed allenylation of Grignard reagent (from **93** to **95**), stereo-selective rhodium-catalyzed C-N coupling between allene and 4-bromopyrazole (from **95** to **96**), hydroboration-oxidation of alkene (from **96** to **97**), Swern oxidation of primary alcohol (from **97** to **98**), oxidative conversion of aldehyde to cyanide in the iodine-ammonia system (from **98** to **99**), borylation of bromo-aromatic derivative using pinacolborane in the presence of palladium(II) catalyst (from **99** to **100**) and Suzuki-Myaura reaction between obtained pinacolborane derivative and 4-chloro-7*H*-pyrrolo[*2,3-d*]pyrimidine attaining desired product, **111** [94]. It is presented in Scheme **18**.
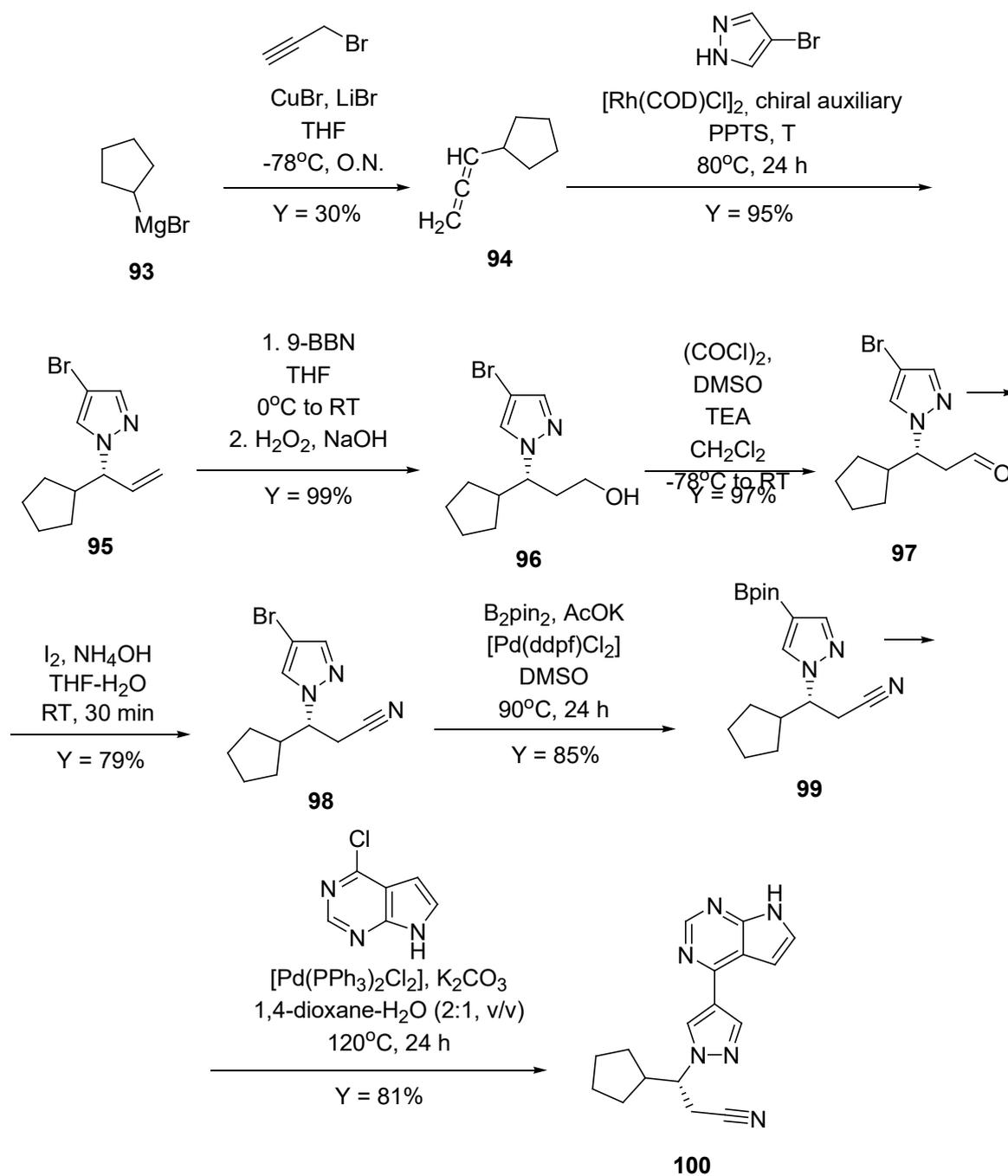
Scheme 18. Multi-step synthesis of Ruxolitinib, **100**.

4.1.3. Isoxazole (Leflunoamide)

Leflunomide (**105**, LF) is classified as an immunomodulator and is categorized among disease-modifying antirheumatic drugs (DMARDs). Preclinical studies have demonstrated that LF possesses immunosuppressive, antirheumatic, antineoplastic, and antiviral properties, suggesting its versatile therapeutic potential. However, clinical studies examining LF's effectiveness in treating viral infections such as cytomegalovirus and BK polyomavirus have yet to yield unequivocal outcomes [95].

LF influences T and B lymphocytes by constraining antibody production thus manifesting antiproliferative activity. Its primary mechanism of action involves the direct inhibition of pyrimidine synthesis by targeting the enzyme dihydroorotate dehydrogenase (DHODH). These lymphocytes rely exclusively on the *de*

novo pathway for pyrimidine biosynthesis, making them vulnerable to the depletion of nucleotide precursors essential for DNA and RNA synthesis. The *in vivo* mechanism of LF's action may vary depending on factors such as drug doses, available uridine pools, and the specific immune activation pathway involved. Studies have suggested that besides inhibiting DHODH, LF and its analogues may also exert their effects by inhibiting tyrosine kinases [88].

One of its synthetic routes, shown in Scheme **19**, involves the functionalization of ethyl acetoacetate, cyclization of the intermediate to isoxazole, and secondary coupling of the isoxazole product with aniline deriver. In the first step, ethyl acetoacetate, **101**, is converted to its methylene-ethoxy derivative, **102**, with triethoxymethane and acetic anhydride. Then, it is cyclized to isoxazole-4-carboxylic acid ester derivative, **103**, using a hydroxylamine aqueous solution. The **103** undergoes hydrolysis in an acidic medium giving free carboxylic acid, **104**. Eventually, acid chloride is generated *in situ* in the thionyl chloride/DMF system and then, this intermediate is condensed with 4-trifluoromethylaniline giving off the desired product, **105** [96].
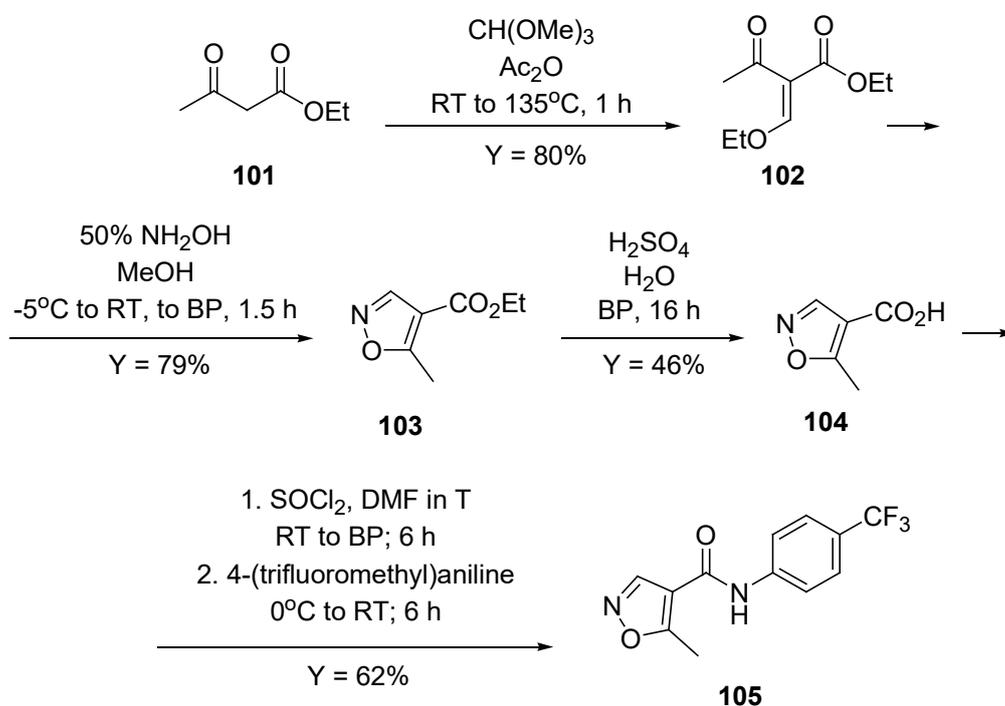
Scheme 19. The chosen method of obtaining Leflunomide, **105**.

4.1.4. 1,2,4-Oxadiazole (Cenerimod)

Systemic lupus erythematosus is an autoimmune disease characterized by the production of harmful autoantibodies, the migration and accumulation of lymphocytes into tissues and organs where they are not normally found (lymphocytes infiltration), and tissue damage in multiple organs. B and T lymphocytes both play pivotal roles in the development of this disease. T lymphocytes not only directly contribute to organ damage but also support the activation and growth of B lymphocytes, which are responsible for autoantibodies production. These autoantibody-producing B lymphocytes, known as antibody-secreting cells (ASC), include proliferative plasmablasts and plasma cells. ASC, being the main effector of B lymphocytes in autoimmune conditions, is closely associated with disease activity in individuals with active SLE [97].

The primary factor responsible for the movement of lymphocytes from peripheral lymphoid organs into the lymphatic and vascular circulation is the chemotactic gradient of sphingosine-1-phosphate (S1P), which

binds to S1P1 receptors on B and T lymphocytes. S1P1 receptor modulators have been approved for treating multiple sclerosis and have demonstrated positive results in other diseases, highlighting their significance in autoimmune disorders. Blocking the activation of S1P1 receptors on the surface of lymphocytes prevents efficient lymphocyte egress from lymph nodes and, consequently, their migration to inflamed tissues. This condition leads to a reduction in the immune response within the tissue, inhibiting inflammation and decreasing the immune system's overall response. This activity decreases the number of active lymphocytes involved in combating inflammation in the body. This mechanism can be utilized to control the progression of the disease and counteract the effects of SLE [97].

Cenerimod (**116**), a drug candidate currently undergoing Phase 3 confirmatory study [98], has demonstrated the ability to reduce blood lymphocytes in mice, which led to decreased accumulation of immune cells in tissues, improved tissue health, reduced protein presence in the urine, and decreased inflammation. As a result, mice treated with Cenerimod exhibited increased survival rates. It effectively promotes the internalization of S1P1 receptors in the lymphocytes of both, healthy individuals and patients with SLE. Additionally, it improved the overall health and inflammation associated with SLE in a mouse model [98].

Cenerimod may be synthesized through a dehydrative cyclization reaction between carboxylic acid and amidoxime component, with the final protective group removal on the asymmetric unit in the side chain. The entire course of the synthesis is presented in Scheme **20**.
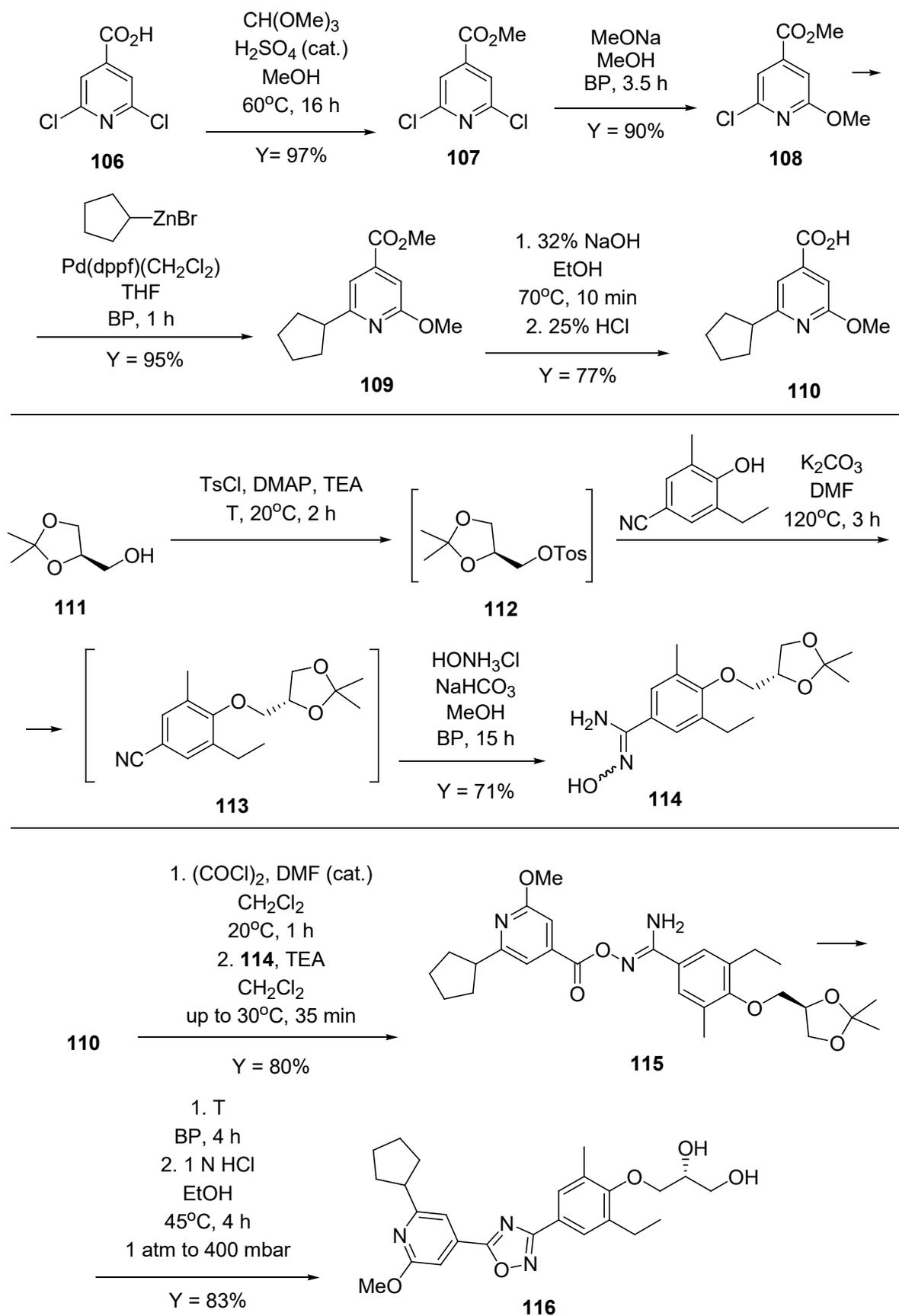
Scheme 20. Preparation of Cenerimod (**116**).

The first pathway considers particular carboxylic acid obtainment. To start with, one may transform 2,6-dichloroisonicotinic acid, **106**, into its methyl ester *via* Fischer esterification protocol in the presence of water scavenging molecules — trimethoxymethane. Then, **107** is treated with sodium methoxide in methanol to achieve a mono-methoxylated product, **108**, obtained *via* nucleophilic substitution. The resulting monochlorinated derivative, **108**, is then substituted using organozinc compound — cyclopentylzinc bromide, the Rieke-type organometallic compound, in the presence of palladium catalyst, providing **109**. Eventually, one may release free carboxylic acid utilizing basic hydrolysis of methyl ester, with further acidification, resulting in **110** formation.

The second pathway assumes the synthesis of amidoxime equivalent. Initially, one may transform glycerol asymmetric deriver, **111**, into its *O*-tosylated form, **112**. Then, without intermediate isolation, one performs the Williamson etherification protocol between obtained **112** and 3-ethyl-4-hydroxy-5-methylbenzonitrile, resulting in **113** formation. Again, without isolation, one transforms **113** into **114** with the use of hydroxylamine hydrochloride in the presence of sodium bicarbonate, reached by nucleophilic addition to the nitrile group.

In the end, **110** and **114** are condensed using the oxalyl chloride/DMF system, giving out **115** as a product. Dehydrative cyclization in boiling toluene with subsequent protective group removal, from the asymmetric part of the molecule, results in the target molecule (**116**) obtaining [99].

4.1.5. 1,3,4-Thiadiazole (TIPTP)

Rheumatoid arthritis is a chronic, inflammatory autoimmune disease associated with oxidative stress, which can cause significant health issues. Reactive oxygen species (ROS), including free radicals such a superoxide and hydrogen peroxide, play a crucial role in the development of RA. ROS are primarily produced by the NADPH oxidase complex (NOX), a group of enzymes involved during inflammatory responses. In RA, elevated levels of ROS have various effects, including oxidation of Immunoglobulin G (IgG) leading to the production of rheumatoid factor, reduce joint viscosity by depolymerizing hyaluronic acid, and impair T cell responsiveness by affecting protein stability and degradation. Increased ROS production can damage cartilage and contribute to bone resorption. Studies in RA patients have shown intensified lipid peroxidation and the presence of oxidative damage markers in synovial fluid and tissues. It is suggested that controlling the production of ROS could be a promising therapeutic approach for treating RA. While ROS are known to play a role in the development of RA, the specific molecular targets responsible for these effects in RA have not yet been fully identified [100].

It has been discovered that the interaction between Rubicon, a protein involved in autophagy, which helps cells remove and recycle damaged components, and p22phox, a subunit of NADPH oxidase (NOX), is a key factor in the production of ROS and the development of RA. Researchers found that a peptide-mimetic compound known as N8, derived from p22phox, effectively inhibits this interaction, leading to the suppression of ROS production and inflammatory cytokines. Subsequently, a small-molecule p22phox inhibitor TIPTP (**122**) was developed, which has significantly improved potency and selectivity compared to a previously reported N8 peptide-mimetic, as it addressed issues related to stability, bioavailability, and metabolic ability when administered *in vivo*. Small molecule compounds have advantages over peptides and monoclonal antibodies in therapeutics development, since they can easily cross cell membranes, are cost-effective, have the potential for oral administration, allow for easier dose adjustment, and are generally associated with fewer immune reactions.

Therefore, **TIPTP** provides a unique resource for the development of a selective therapeutic option for RA treatment [100].

The synthesis of **122** was carried out starting with tetrahydroindazole, **117**, which was coupled with 3-methoxyiodobenzene *via* Ullmann-type coupling catalysed with copper(I) chloride in the presence of ligand and base. Then, the deetherification protocol of **118** was implemented using boron tribromide in dichloromethane, resulting in **119** formation. The phenolic compound, **119**, then acts as a nucleophile with methyl (*S*)-2-chloropropionate, in the presence of base, delivering **120**. Deprotection protocol of an ester moiety, under lithium hydroxide conditions, was then implemented thus giving **121** as a free carboxylic acid. The latter was then condensed with 2-amino-5-trifluoromethyl-1,3,4-thiadiazole, utilizing the hydroxybenzotriazole-uronium system in the presence of tertiary amine, giving the final product, **122**. The target molecule was obtained with a slightly over 6% yield [100]. Its synthesis is outlined in Scheme **21**.
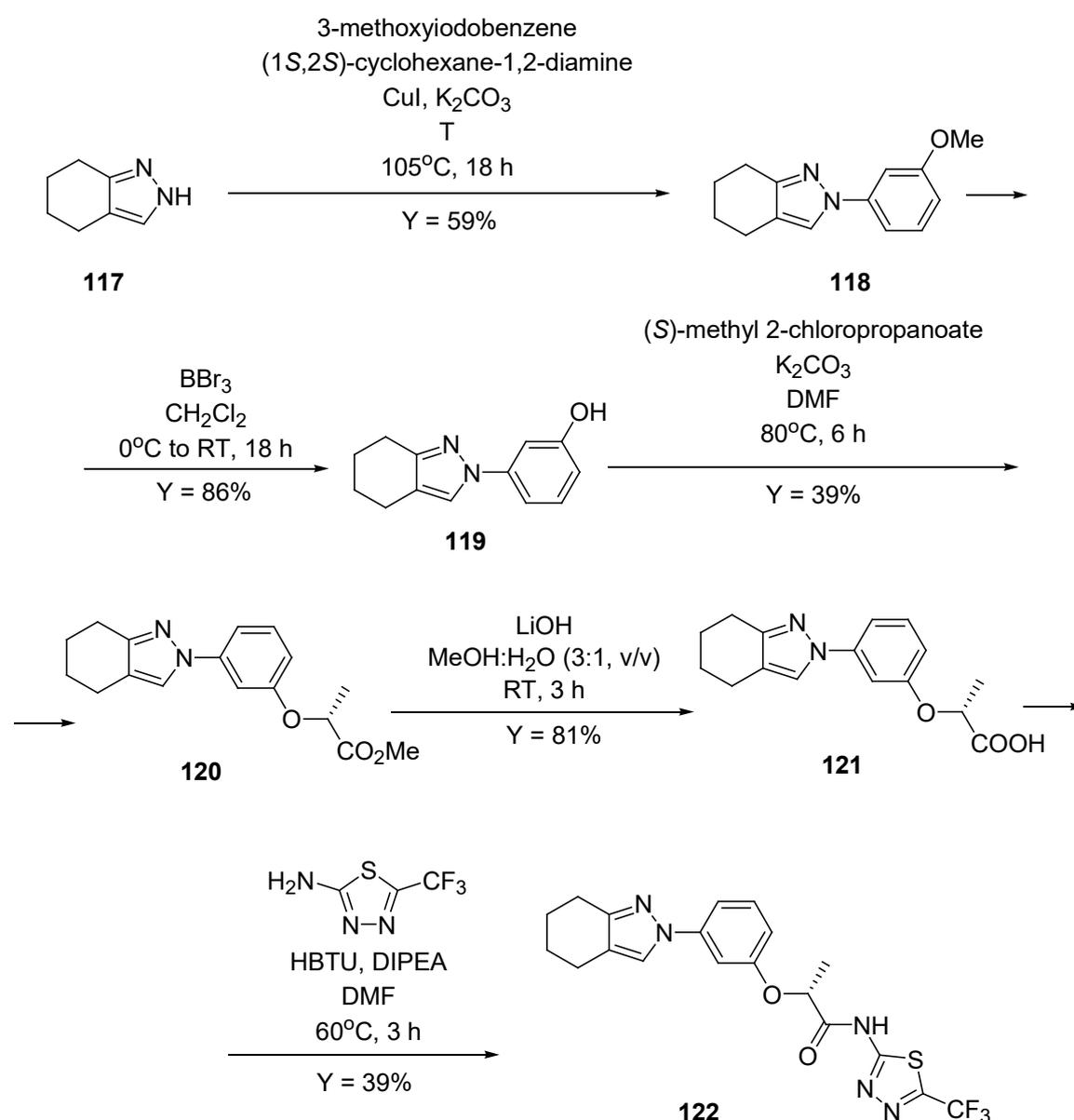
Scheme 21. TIPTP (**122**) synthesis conspectus.

4.2. 6-membered rings

4.2.1. Pyridine (BZS_1)

The immune system has special cells called cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells to maintain a balanced immune response. These cells use a process called granule exocytosis, a cellular mechanism enabling the rapid release of specialized molecules, to eliminate virus-infected and cancerous cells. When a CTL or NK cell recognizes a target cell, they form a structure called an immunological synapse. Within this synapse, secretory granules move to the surface of the immune cell and release their contents into the space between the two cells. This exposes the target cell's membrane to two substances: perforin, which forms arc- and ring-shaped pores, and granzymes, a group of serine proteases. Together, these substances efficiently destroy the target cell, inducing apoptosis [101].

Perforin, a glycoprotein, plays a crucial role in the process by creating pores in the target cell's membrane. These pores disrupt the osmotic balance of the cell, allowing granzymes to enter and trigger apoptosis. Notably, despite both the effector and the target cell being exposed to perforin within the synapse, only the target cell membrane is disrupted. As a result, cytotoxic effector cells can kill multiple target cells quickly without being harmed. It has been demonstrated that benzenesulfonamide-based compounds (BZS) effectively inhibit CTL/NK cell killing system. Importantly, the immune response is fully restored upon cessation of treatment, indicating the fast and complete reversibility of perforin inhibition. Nevertheless, targeting perforin holds the potential for regulation or inhibition of the immune response making it a promising strategy for developing immunosuppressants and immunmodulators [101].

Benzenesulfonamide (BZS) inhibitors of perforin were synthesized and tested for reducing of graft rejection in bone marrow/stem cell transplantation and effectively inhibit perforin both in *in vitro* and *in vivo* conditions. Among them, only compound **127** was evaluated for its effectiveness in preserving allogeneic bone marrow. The study revealed a strong relationship between perforin inhibition and compound **127** concentration in the blood, with effective levels maintained for a longer duration than in laboratory tests. This approach will maximize the effectiveness of the treatment and advance its progress towards clinical evaluation [101].

The strategy of obtaining BZSs comprises a series of Suzuki reactions between different halo-compounds and boronic acids or their esters, as well as the eventual nucleophilic substitution in an acyl system providing *N*-acylated final product, **127**. Its preparation is shown in Scheme **22**.
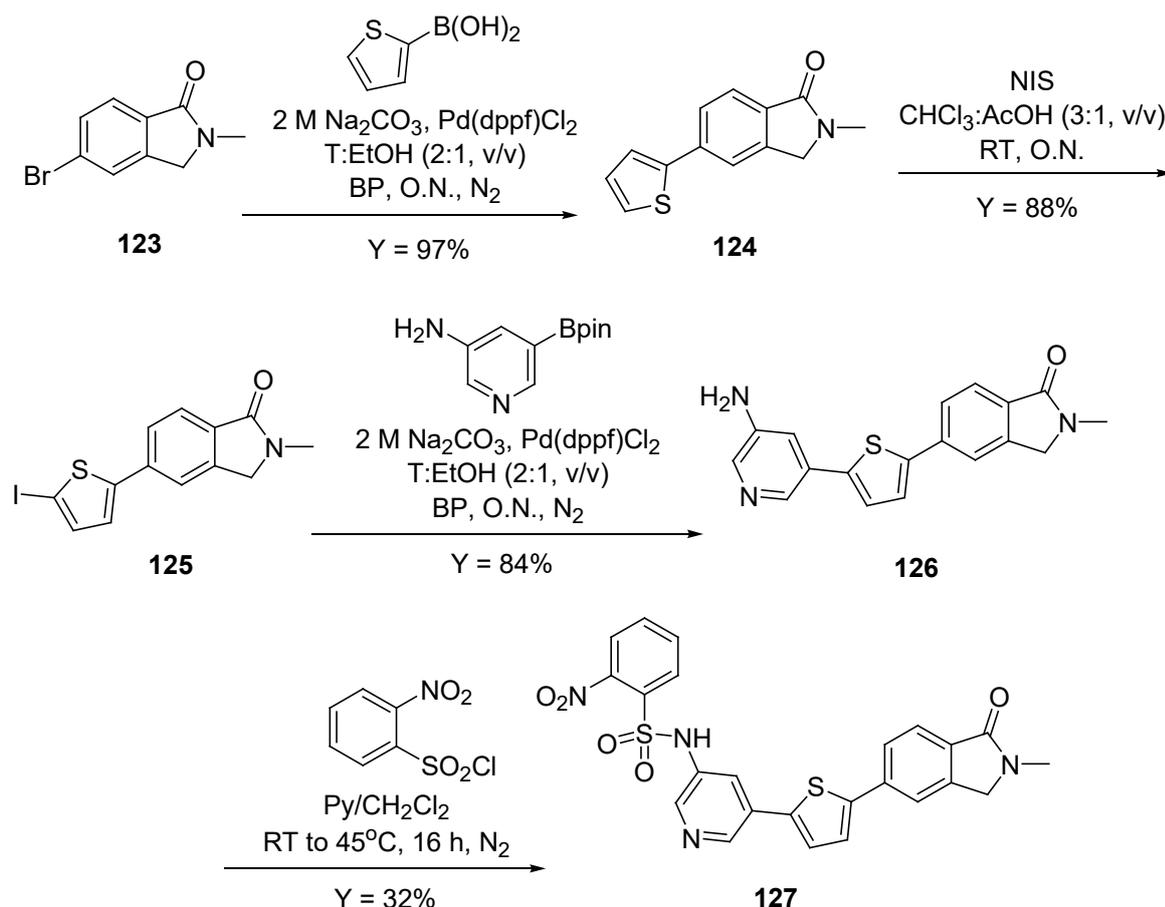
Scheme 22. The synthetic route for **127** obtainment.

In the beginning **123**, *N*-methylisoindolin-1-one commercially available deriver, was coupled with thiophene-derived boronic acid, in the presence of a base and Pd(II) catalyst, resulting in **124** formation. Then, the thienylated product was iodinated using *N*-iodosuccinimide to obtain a 2-iodothiophene structural motif, here: **125**. Again, the Suzuki reaction protocol was implemented to achieve **126** as an aminopyridine deriver. In the end, the remaining free amino group was acylated using 2-nitrobenzenesulfonyl chloride, in the presence of pyridine, to acquire the target molecule, **127**. This simple reaction protocol facilitates functionalized **127** derivatives obtainment [101,102].

4.2.2. Pyrimidine (Evobrutinib)

Multiple sclerosis is a common autoimmune disease that frequently results in neurological disability, primarily in young adults. It is characterized by inflammation, loss of protective covering around nerve fibres, increased brain cell growth, and nerve damage. The disease only affects the central nervous system (CNS), sparing the peripheral counterpart. There are two main forms: relapsing or progressive. The most common form is relapsing MS (RMS), which involves episodes of neurological dysfunction followed by periods of partial or complete recovery. Over time, these episodes become less frequent, but the condition generally worsens, leading to uninterrupted progression. All forms of MS share the same disease aetiology with inflammation and nerve degeneration present in all patients, regardless of the disease stage [103].

Bruton's tyrosine kinase (BTK) is a protein found in B cells, macrophages, and microglia - specialized immune cells found in the CNS that regulate inflammation and tissue repair. Elevated BTK levels have been observed in B cells of relapsing-remitting MS and secondary progressive MS patients, as well as in microglia within progressive MS lesions. This makes BTK an attractive target for autoimmune disease therapy, and BTK inhibitors are being investigated for diseases such as MS, rheumatoid arthritis, and systemic lupus erythematosus [104].

Evobrutinib (**132**) is an oral medication that specifically inhibits BTK and can penetrate the CNS. It is currently undergoing phase III clinical trials for MS. Additionally, **132** has also been studied in phase II trials for RA and SLE and have shown that is well-tolerated, with no observed dose-related adverse events. Safety analyses based on a large database of over 1000 patients who participated in three phase II trials for various autoimmune conditionshave confirmed that evobrutinib is well tolerated in patients with MS, RA, and SLE. These findings may enhance one's understanding of evobrutinib's safety profile and support its continued development for MS, including the ongoing phase III trials [104].

Evobrutinib may be obtained *via* pyrimidine structural motif functionalization, carried out through nucleophilic substitution, Suzuki coupling, protective group removal and nucleophilic substitution in an acyl system. This method is presented in **Scheme 23**.
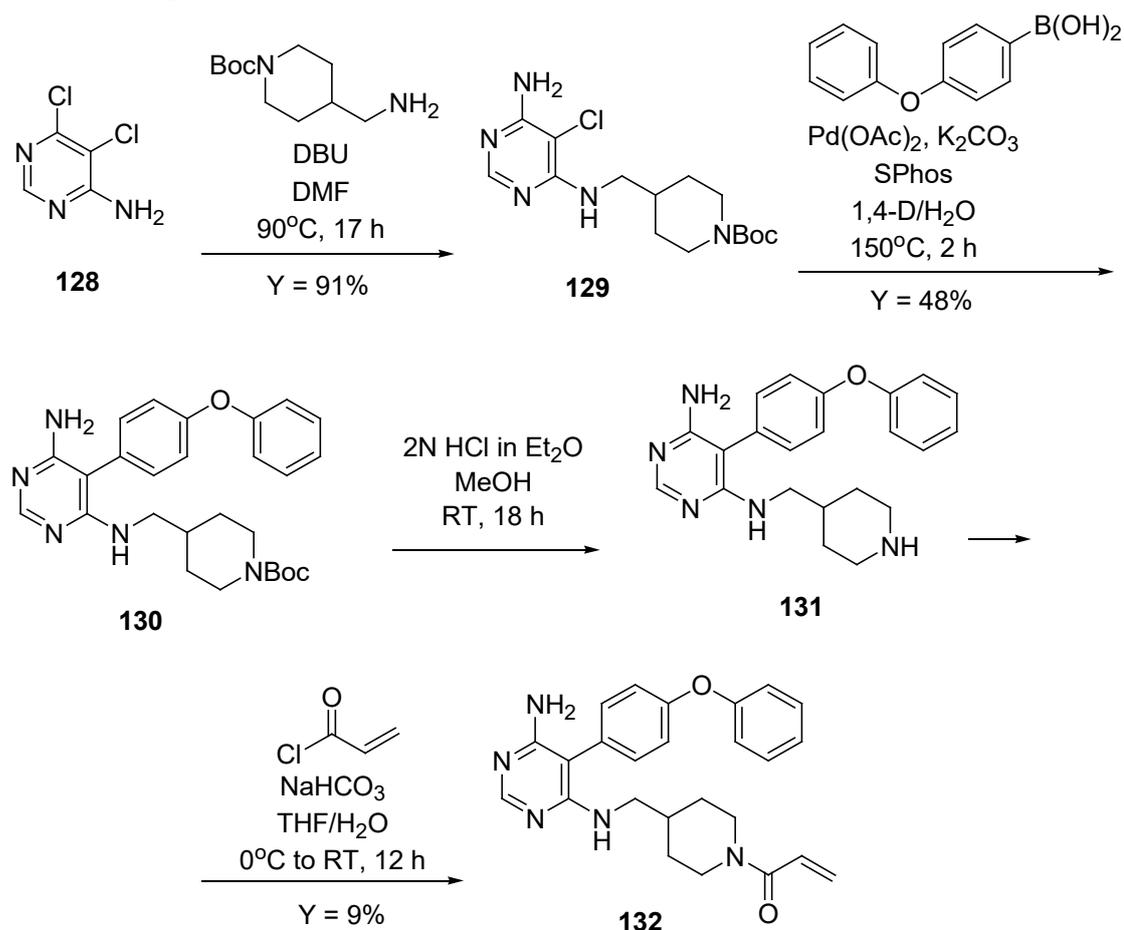
Scheme 23. Synthetic pathway leading to Evobrutinib (**132**).

In the first step, one may proceed with 5,6-dichloropyrimidin-4-amine, **128**, nucleophilic substitution using *t*-butyl 4-(aminomethyl)piperidine-1-carboxylate to obtain **129** as monosubstituted product. Then, utilizing Suzuki reaction methodology, one may couple **129** with particular boronic acid, in the presence of palladium(II) salt, base and auxiliary phosphine ligand, resulting in **130** formation. Then, the amino protective group (*N*-Boc) in **130** can be removed using etherified hydrogen chloride thus giving out **131**. In the end, free secondary amine **131** (without purification) can be acylated using acryloyl chloride, in the presence of base, driving this synthetic pathway to completion (**132**) [105].

4.2.3. 1,3,5-Triazine (**133**, **134** and **135**)

The connection between cancer and immune function remains unclear, however some limited advancements in this field have been made. It is widely understood that cancer cells have developed strategies to evade the immune system, and it may be achieved through various mechanisms involving immunosuppressive cytokines and immune checkpoint receptors (ICRs). These molecular mechanisms contribute to the alteration of the tumour microenvironment (TME) locally and create conditions in secondary organs that promote the formation of pre-metastatic niches. These niches create an ideal environment for immune evasion and uncontrolled growth of cancer cells.

It is also indicated that exosomes, cell membrane-anchored extracellular vesicles, presumably responsible for intercellular communication, released by tumours play a crucial role in suppressing the overall antitumor immune response systemically [106].

Among various ICRs such as: cytotoxic T-lymphocyte protein 4 (CTLA4), programmed cell death protein 1 (PD-1) and indoleamine 2,3-dioxygenase (IDO). PD-1, a receptor found on the surface of activated CD8$^+$ T cells, is of particular interest. It has been discovered that within the TME the expression of PD-1 on infiltrated T cells can increase. Simultaneously, the ligand for PD-1, PD-L1, is overexpressed on the membranes of tumour cells and extracellular vesicles. The upregulation of the PD-1 receptor on lymphocytes makes them more susceptibility to suppression because this receptor serves as an inhibitory regulatory signal in the immune response. When a cancer cell releases cytokines like TGF-β and IL-10, it results in elevated PD-1 receptor expression on lymphocytes. The binding between receptor and ligand generates an inhibitory signal that dampens the activity of T lymphocytes, reducing their effectiveness in combating cancer cells. Ultimately, this leads to the suppression of the antitumor immune response and increases the likelihood of T lymphocyte suppression or apoptosis [106].

A series of 2,4,6-tri- and 2,4-disubstituted 1,3,5-triazines were synthesized and tested using qualitative 1D ^{1}H NMR analysis. Additionally, quantitative testing was conducted using a homogeneous time-resolved fluorescence (HTRF) binding assay, which provided an IC$_{50}$ value for each compound in the context of the PD-1/PD-L1 system inhibitory properties evaluation. Three of them, namely: **133**, **134** and **135** exhibited the most promising values: 0.115, 0.241 and 0.315 μM, respectively. Among them, the disubstituted triazine **133** revealed the highest potency. Further NMR analysis demonstrated its specific binding to PD-L1 but not PD-1. The binding event was also confirmed through NMR assays conducted on PD-L1-containing exosomes. The results of immunofluorescent double-staining assay showed that **133** effectively binds to PD-L1 on cell membranes, leading to the restoration of peripheral blood mononuclear cells (PBMCs) function when co-cultured with lung adenocarcinoma cells (PC9 and HCC827). Treatment with **133** resulted in increased secretion of IFN-γ, an

immune-modulating molecule, and enhanced induction of cell death in the cancer cells (PC9 and HCC827) [106].

133's synthetic route starts with commercially available 2,5-dichloro-1,3,5-triazine (DCT, **136**) which chlorine atom undergoes nucleophilic substitution with appropriate *O*- and *N*-nucleophiles. It has been assumed that the first substitution of the chlorine atom (in DCT) takes place at room temperature, and the second at about 70ºC. In the first step, (2-methyl-[1,1'-biphenyl]-3-yl)methanol reacts with DCT in the presence of tertiary amine, in dichloromethane, giving monosubstituted product, **137**. Then, it undergoes another substitution using *N*-(2-aminoethyl)acetamide yielding **133**. Overall yield of the given method equals 24% [106]. The aforementioned transformations are presented in Scheme **24**.
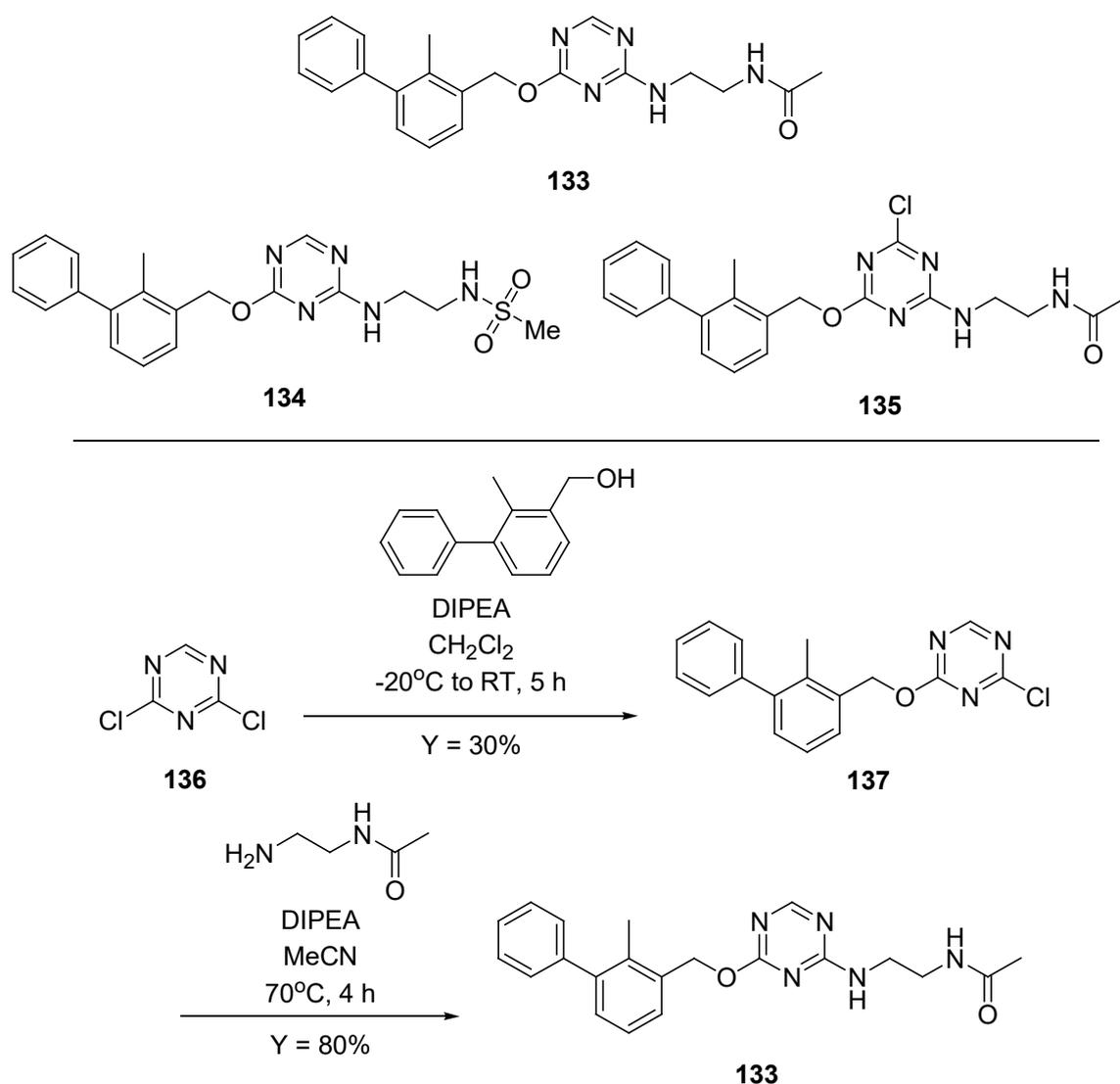
Scheme 24. Structures of 1,3,5-triazine derivatives and preparation of derivative **133**.

1,3,5-Triazine derivatives example may contribute to the understanding of immunosuppression mechanisms and provide insights into potential therapeutic approaches for modulating immune responses in the context of cancer and other immunosuppressive conditions. While this is a non-obvious example, presenting

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anti-immunosuppressive properties, it provides an opportunity to identify a key immunosuppressive receptor, identify a class of potential new immunomodulators, and discuss new approaches in immunotherapeutic strategies to overcome immunosuppression in cancer treatment.

4.2.4. Piperidine (Tofacitinib)

Systemic sclerosis (SSc) is a rare autoimmune disease that affects connective tissues and multiple organs. It is characterized by fibrosis, thickening and scarring of tissues, and vasculopathy, abnormal blood vessel function. Advancements in the treatment of SSc, particularly in early diffuse cutaneous SSc (dcSSc), have been supported by evidence from randomized clinical trials. In SSc, the dysfunction of the immune system leads to the activation and increased numbers of T cells, B cells, macrophages, and dendritic cells. These cells release multiple cytokines and produce auto-antibodies which in turn contribute to the development of fibrosis. Regulation of immune cells and specific cytokines inhibition may serve as a foundation for fibrogenic process reversal [107,108].

Cytokines, which are small proteins or peptides that act as chemical messengers, play a crucial role in regulating how fibroblasts, a type of connective tissue cells that provide structural support and organization to the surrounding cells, , build the extracellular matrix (ECM). Once cell surface receptors are activated by binding cytokines, a group of tyrosine kinases known as JAK, including JAK1, JAK2, JAK3, and TYK2, initiate the translocation of signal transducers and activators of transcription (STATs) to the cell nucleus. This translocation of STATs ultimately regulates the transcription (gene expression) of type I and type II cytokines.

JAK-STAT signalling is a critical mechanism that coordinates immune system responses and has a crucial role in various autoimmune disorders triggered by cytokines. Inhibiting JAK, can lead to a decrease collagen production and the effects of transforming growth factor-β (TGF-β) stimulation in fibroblasts associated with systemic sclerosis SSc. Therefore, JAK hampering has been recognized as a potential therapeutic approach in different research studies, especially those concerning autoimmunity [107-109].

Tofacitinib (**147**) treatment reduced skin and lung fibrosis in a mouse model of scleroderma induced by bleomycin, a chemotherapeutic agent used to mimic the fibrotic characteristics observed in the SSc. It also suppressed the immune responses by decreasing certain immune cells and molecules, such as: splenocytes, total lymphocytes, T helper cells, IL-6-producing B cells and certain types of macrophages in the skin and lungs. Conversely, it increased the relative numbers of T and B cells in the spleen. Tofacitinib also lowered the expression of extracellular matrix proteins and fibrogenic cytokines in both the skin and lungs [108].

Tofacitinib may be synthesized using a coupling reaction between two key intermediates: optically active piperidine derivative and *N*-methyldeazapurine. Both of them can be obtained from commercially accessible substrates.

To start with, one may treat 4-piperidone, **138**, with benzyl bromide in the presence of a base, resulting in *N*-benzylated product, **139**, formation. Then, using α-aminoxylation reaction protocol, with nitrosobenzene as the N-O bond providing molecule, catalyzed with chiral auxiliary — L-proline, and Wittig reaction, one can obtain **140** as *N,O*-substituted hydroxylamine deriver. N-O bond cleavage can be carried out under Cu(II) ions conditions with allylic alcohol, **141**, release. Asymmetric hydrogenation of alkene on palladium catalyst gives out **142** with the *N*-protective group remaining unimpaired. **143** was obtained via mesyl chloride treatment on **142** in the presence of a base.

Secondarily, **146** was acquired by carrying out a nucleophilic substitution of the chlorine atom in 4-chlorodeazapurine, **145**, with an ethanolic solution of methylamine at elevated temperature and pressure.

In the end, an asymmetric unit (**143**) was installed in *N*-methyldeazapurine, **145**, via nucleophilic substitution in the presence of a base. Obtained **146** was then treated with Pd(II)/TFA/hydrogen gas system to remove the *N*-protective group. *N*-Acylation of the resulting intermediate was performed using 2-cyanoacetic chloride in the presence of a tertiary amine. Enantioselective synthesis of **147** showed 18% overall yield and high enantiomeric excess — 96.8% [110]. The entire synthetic pathway is shown in Scheme **25**.
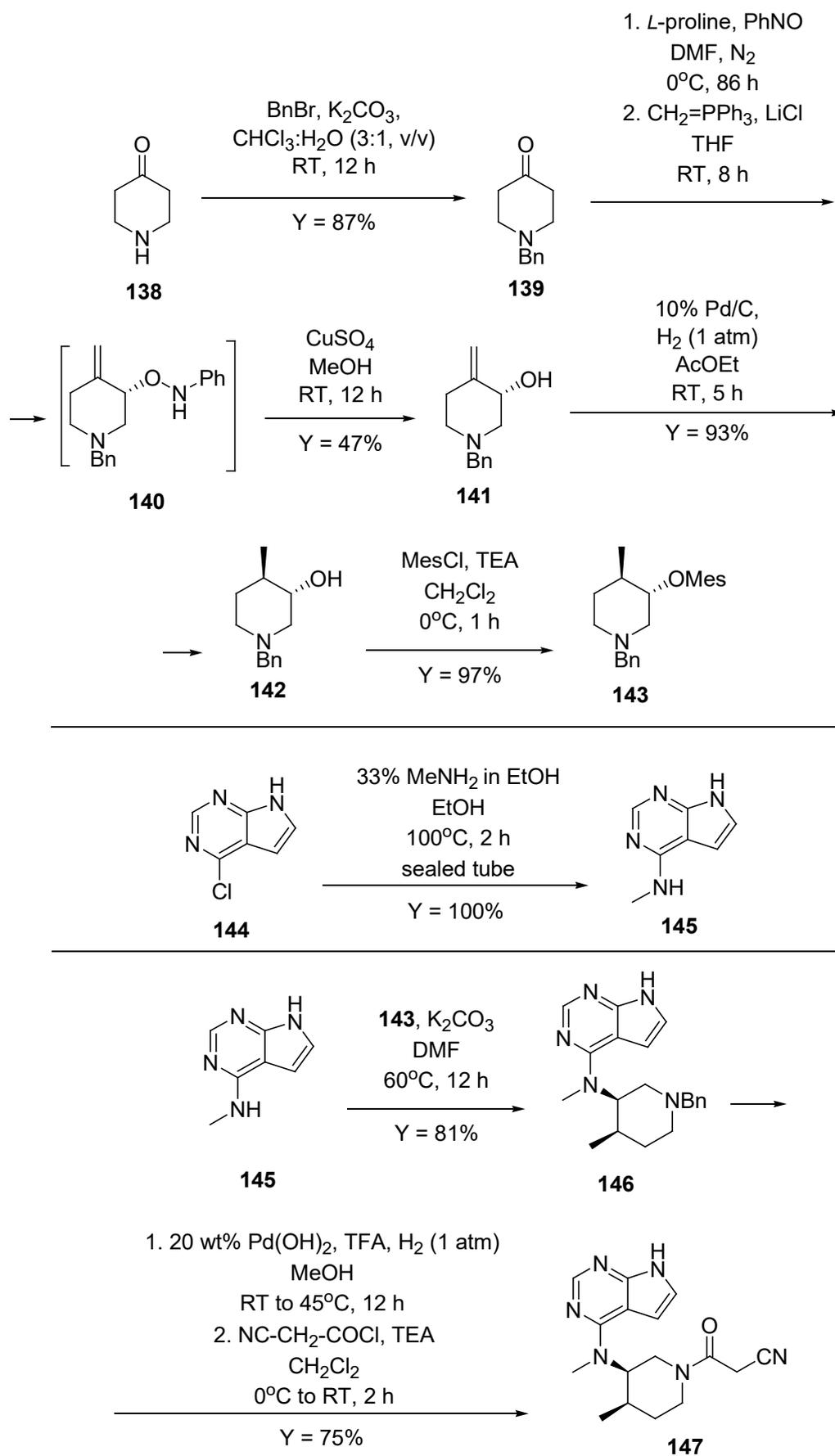
Scheme 25. Tofacitinib (**147**) synthesis route.

4.2.5. Piperazine (Lanraplenib)

Spleen tyrosine kinase (SYK) is a crucial component of the immune cell signalling system through various receptors in different immune cell types, including the B cell receptor (BCR), Fc receptors (FcR), and β2 integrin. Inhibiting or removing SYK can have significant negative effects on the immune system since this protein is intricately involved in the development, activation, and maturation of B cells, as well as antibody production and class switching. Dysregulation of SYK has been observed in patients with SLE and in the lupus nephritis (LN), which is a kidney manifestation of SLE, and is characerized by the accumulation of cells expressing SYK in the kidney's filtering units. While inhibitors of SYK have been developed and tested in animal models of lupus, none of them have undergone clinical trials involving human patients with SLE or LN [111].

Lanraplenib (**154**) underwent a comprehensive biological evaluation, encompassing several key aspects: its ability to inhibit B cell functions in human cells, its effectiveness in reducing LN-like disease on New Zealand black/white mice model (ZNZB/W) in relation to control substances, its impact on kidney health which was assessed measuring glomerulopathy indicators and the deposition of IgG. Additionally, the evaluation included the analysis levels of pro-inflammatory cytokines in the blood, B cell, and T cell maturation by splenocytes analysis, and the assessment of the presence of T follicular helper and dendritic cells, which serve as immune response regulators and initiators. I The results demonstrated that lanraplenib effectively halted the progression of LN-like disease in the *in vivo* mice model due to improved overall survival, prevention in the development of proteinuria and reduction of blood urea nitrogen levels. Both human cell culture and mouse experiments indicate that lanraplenib may be useful in slowing down the progression of LN in patients, potentially by targeting B cell maturation [112].

Lanraplenib was obtained using a series of nucleophilic substitutions in an aromatic system, *N*-protective group incorporation, and Suzuki reactions. All of them are summarized in Scheme **26**.
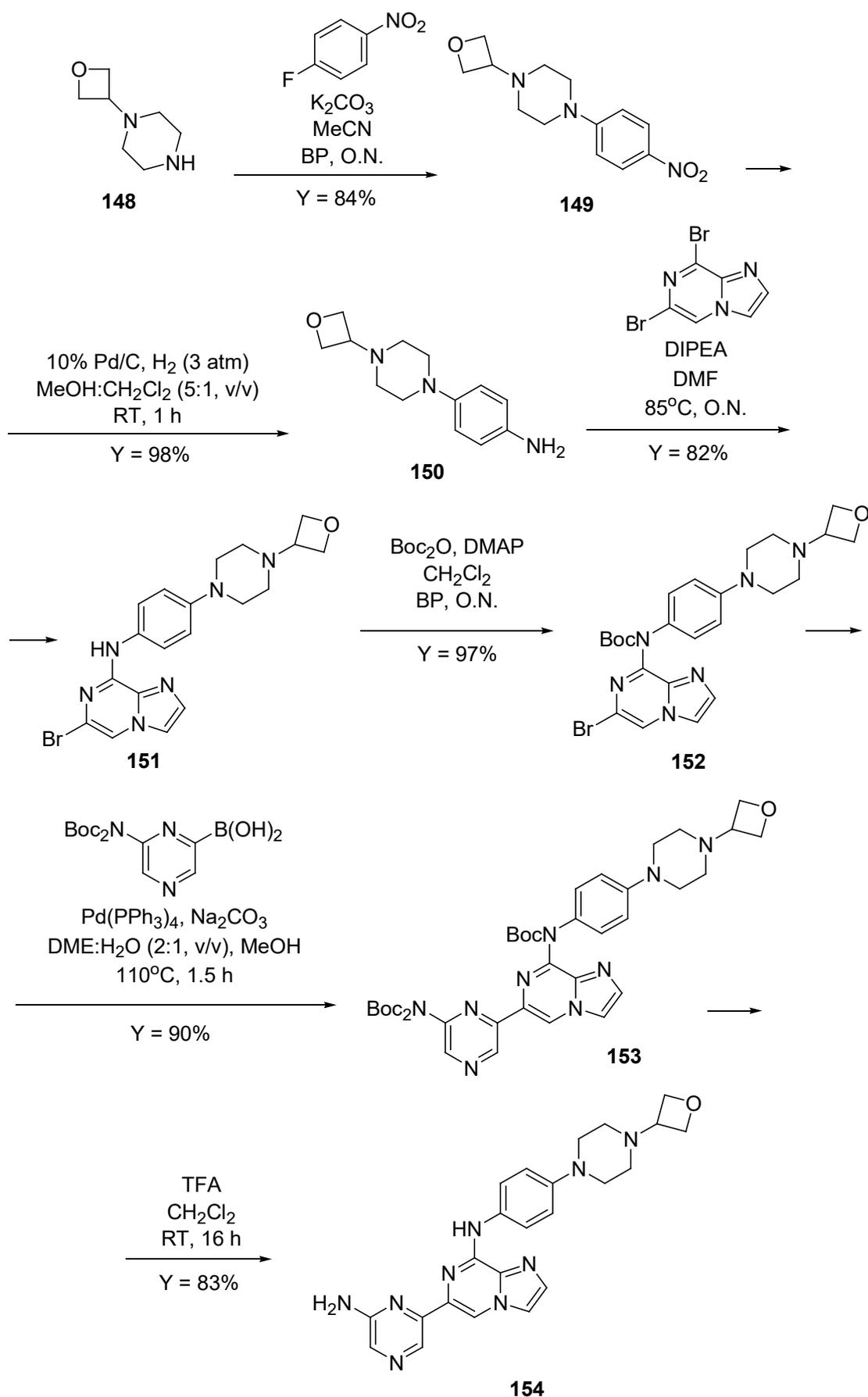
Scheme 26. Synthesis of piperazine derivative, **154,** as SYK inhibitor.

In the beginning, **148** was treated with 4-fluoronitrobenzene, in the presence of a base, to afford **149**. Obtained nitro-derivative was then reduced using hydrogenolysis under catalysis of palladium on carbon, resulting in the **150** formation, as free amine. Then, 6,8-dibromoimidazo[1,2-a]pyrazine was treated with **150**, in the presence of DIPEA, to afford **151**. The remaining secondary amino group was protected using Boc$_2$O with the catalysis of DMAP to acquire **152**. The Suzuki reaction was implemented to obtain **153** as a pyrazine deriver. Final acidic deprotection of *N*-Boc groups allows one to get the target molecule, **154**. The total yield of Lanraplenib is 49% [112].

4.3. Fused rings

4.3.1. Benzoxazole-derived compounds

1,3-Oxazole is an important structural motif amid various bioactive compounds, encompassing chemotherapeutic agents, antimicrobials, neuroprotectives, and analgesics. These structural cores derived from 1,3-oxazole may be found in drugs used for the treatment of cardiovascular diseases and as inhibitors of carbonic anhydrase (isoforms I and II). Furthermore, 1,3-oxazole derivatives have demonstrated anti-inflammatory activity by inhibiting key enzymes such as glycogen synthase kinase 3 β (GSK3β), a member of the serine/threonine protein kinase family that plays a vital role in regulating essential cellular processes, including glycogen metabolism, cell signalling, gene expression, cell cycle progression, and cell survival.. They also inhibit mitogen-activated protein kinase p38 α (MAPK p38α), a critical enzyme that plays a central role in cellular signalling, mediating responses to various external stimuli and influencing diverse cellular processes. Additionally, these derivatives maintain the enzyme activities of COX-2 and COX-1, two isoforms of the enzyme cyclooxygenase which play key roles in the production of prostaglandins — compounds responsible for proper inflammatory responses in the body. Some oxazole derivatives have been found to inhibit T-cell proliferation through the JAK3/STAT5 signalling pathway. However, the immunosuppressive activity of 1,3-oxazole derivatives via SGK1 inhibition has not been previously reported [113].

SGK1 also known as serum and glucocorticoid-regulated kinase 1 or Serine/threonine-protein kinase, is a protein kinase regulated by serum factors and glucocorticoids. It plays a pivotal role in various cellular processes, including cell survival, proliferation, differentiation, and ion transport. SGK1 is instrumental in mediating the effects of glucocorticoids on gene expression and cellular responses. In the context of immunosuppressive properties, SGK1 in known to modulate immune cell functions by affecting the activity of transcription factors, such as NF-κB (Nuclear Factor kappa-light-chain-enhancer of activated B cells, a protein complex that plays a critical role in activation or repression of the expression of target genes which regulate cell survival and apoptosis), and regulating the expression of cytokines and other immune-related molecules. SGK1 inhibition may serve as a foundation for therapeutic strategies in diabetes, inflammations as well as some types of cancers [114].

New compounds featuring the benzoxazole structural core, as shown in Fig. **11**, were developed, and one of them with potent immunosuppressive properties. Among these compounds, **155**, **156,** and **157** exhibit IC$_{50}$ values as follows: 0.7, 15.1, and 24.5 μM, respectively. It turned out that **155** specifically targets and inhibits the activity of SGK1, leading to the inhibition of T-cell proliferation. In preclinical studies using a mice model, **155** showed effectiveness in reducing dinitrofluorobenzene-induced delayed-type hypersensitivity reaction and

imiquimod-induced dermatitis. However, further research is needed to explore the structure-activity relationship of **155** and optimize its properties for treating organ transplant rejection and autoimmune diseases [113].
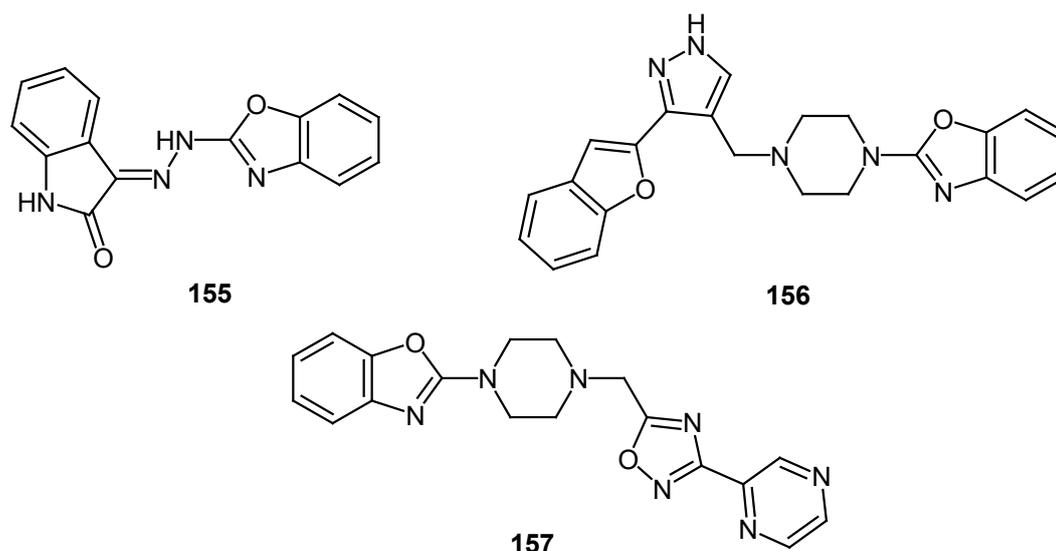
Fig. (11). Structural formulas of SGK1 inhibitors (**155-157**) containing the benzoxazole system within their structure.

4.3.2. Pyrimidine-fused bicyclic derivers

Pyrimidine-fused bicyclic heterocycles are thought to serve as promising frameworks with valuable bioactivities in diseases such as: cancer, viral infections, inflammation, cardiovascular issues, erectile dysfunction, diabetes, and parasitic infections due to their presence during numerous drugs design for given disorders. Among 147 pyrimidine-fused bicyclic heterocycles, 57 derivatives had been approved for clinical treatment of different diseases and 90 of them were in various stages of clinical development (in 2020). Out of the 57 approved drugs, 22 had been specifically approved for the treatment of different cancers. Some of them had been investigated for EGFR (Epidermal Growth Factor Receptor) and PI3K (Phosphoinositide 3-Kinase) inhibition [115].

EGFR and PI3K are signalling pathways that play important roles in cell growth, survival, and proliferation [116-119]. They can affect immune system responses secondarily since their primary function is not directly related to immunosuppression. EGFR signalling in certain cancers can lead to immunosuppressive effects within the tumour microenvironment. Tumour cells release factors that suppress immune responses, allowing them to evade the immune system. EGFR signalling contributes to this immunosuppression by promoting the expression of immunosuppressive molecules and inhibiting the function of immune cells like T cells [116,117]. PI3K pathway plays a role in cell survival, proliferation, and metabolism. Dysregulation of PI3K signalling in cancer and inflammatory diseases can contribute to immunosuppression. PI3K activation inhibits immune cell function and promotes the development of immunosuppressive cell types like regulatory T cells and myeloid-derived suppressor cells [118,119].

Some of the pyrimidine-fused bicyclic derivatives, highlighted in Fig. **12**, have been positively revised by the Food and Drug Administration (FDA): Dacomitinib (**158**) for non-small cell lung cancer (NSCLC) with EGFR mutation treatment, Tucatinib (**159**) for advanced unresectable or metastatic HER-2 positive breast cancer

therapy as well as Duvelisib (**160**) as a remedy for chronic lymphocytic leukaemia (CLL) and small lymphocytic lymphoma (SLL) [115].

158
Dacomitinib (2018)
EGFR inhibitor

159
Tucatinib (2020)
EGFR inhibitior

160
Duvelisib (2018)
PI3K inhibitor
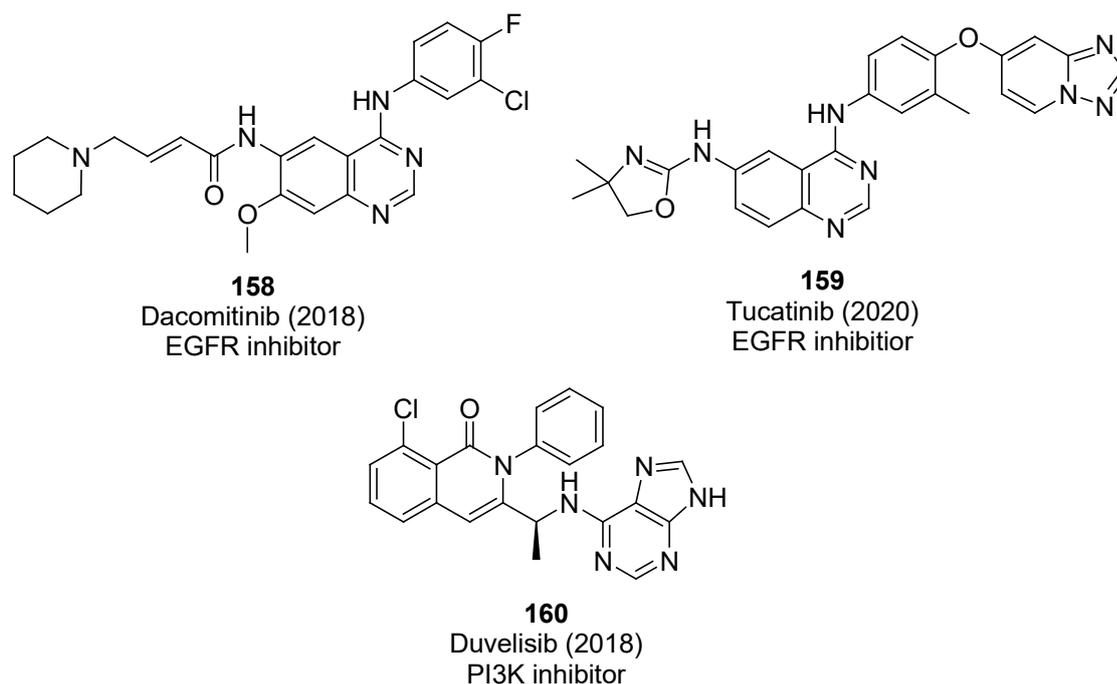
Fig. (12). Structures of EGFR (**158** and **159**) and PI3K (**160**) inhibitors approved for clinical use by the FDA.

4.3.3. Methotrexate derivatives

Methotrexate (MTX, **161**), drawn in Fig. **13**, is a dihydrofolate reductase (DHFR) inhibitor and is applied in clinics as an immunosuppressant in RA, and psoriasis treatment. Due to decreasing nucleic acid synthesis, it is also investigated as a prospective agent against cancer. However, methotrexate revealed low selectivity against cancer cells and causes serious side effects. As a result, methotrexate derivatives were designed as novel active substances to improve therapeutic properties [120].

161
Methotrexate (MTX)
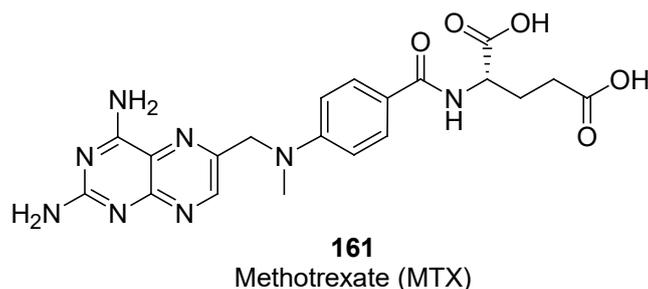
Fig. (13). Structural formula of Methotrexate (**161**).

Recently, complexes of **161** with divalent metals: strontium, magnesium, zinc were obtained and evaluated as prospective improvements of commercially used drugs against RA [121]. This modification increased thermal stability of the drug, glycosaminoglycanes deposition together with *in vitro* NO production decrease, which indicated anti-inflammatory properties maintenance. According to these promising results, the observed activity may provide regeneration of damaged issues in RA treatment and should be further *in vivo* studied.

4.3.4. 6-Mercaptopurine and its derivatives

6-Mercaptopurine (**162**) is an antimetabolite, which interrupts DNA and RNA production leading to apoptosis. One of the stages of these metabolic pathways is a competitive reaction with hypoxanthine-guanine phosphoribosyl transferase (HGPRT) to inhibit purine nucleotides biosynthesis. Due to its antiproliferative properties, 6-mercaptopurine is active against human leukaemia and autoimmune disorders: inflammatory bowel disease, SLE, RA [122,123]. Recently, Pt(II) complexes of 6-mercaptopurine were recorded as compounds improving its bioavailability [124]. The obtained complexes revealed interactions with human serum albumin and gave better cytotoxic activity against the K562 cell line than cisplatin.

A significant prodrug of 6-mercaptopurine is azathioprine (**163**). This imidazole-derived purine is used clinically in autoimmune disease treatment and prevention of renal transplant rejections [125-127]. However, in the case of intolerance to AZA, therapy with its metabolite 6-mercaptopurine can diminish side effects for patients [128]. Both the drug (**162**) and its prodrug (**163**) are shown in Fig. **14**.

162
6-mercaptopurine

163
azathioprine (AZA)
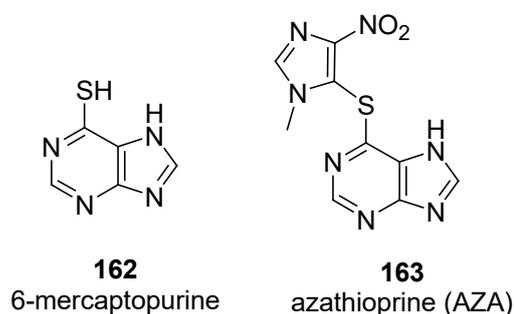
Fig. (14). Structural formulas of compounds based on the 6-mercaptopurine core (**162** and **163**).

4.3.5. Diazepam immunosuppressive properties unveiled

Benzodiazepines are psychoactive drugs with sedative, hypnotic, anxiolytic, and anticonvulsive properties. They are commonly used to induce muscle relaxation and amnesia in anaesthetics. These drugs may interact with both central and peripheral benzodiazepine receptors, resulting in relevant immune system responses. These responses have the potential to be utilized for therapeutic purposes, yet they present both opportunities and difficulties in their effective utilization. Benzodiazepines such as diazepam exhibit immunomodulatory characteristics, capable of hampering exaggerated immune reactions, thereby mitigating inflammation and potentially offering therapeutic benefits in conditions characterized by excessive immune activation, such as autoimmune diseases or septic shock. While suppressing the immune response can be beneficial in preventing tissue damage or in autoimmune diseases, it may also increase the risk of infections or compromise the body's ability to mount an appropriate immune response against pathogens.

The central benzodiazepine receptor is found in the CNS as part of the $GABA_A$ receptor complex, while the peripheral benzodiazepine receptor, known as translocator protein 18 kDa (TSPO, previously referred to as the peripheral benzodiazepine receptor), is present in various cells and tissues including platelets, immune cells, endothelium, and glial cells in the CNS. In CNS diseases like Alzheimer's, Huntington's, and multiple sclerosis, TSPO levels increase due to microglial activation, a key part of the inflammatory response. In general sense, TSPO expression tends to be upregulated in reaction to cellular stress, inflammation, or injury. When microglia, the brain's immune cells, become active, they can trigger neuroinflammation, potentially causing nerve cell damage and degeneration. Consequently, TSPO is being explored as a target for treatment, with its ligands under study for their potential to protect nerves, promote axonal regeneration, and regulate inflammation.

Diazepam (**166**), which is a compound belonging to the benzodiazepines group, turned out to be a potent immunosuppressant that effectively diminished inflammatory immune responses. It impairs antigen presentation and disrupts secondary signals necessary for proper activation and polarization of adaptive immune cells towards inflammatory conditions. Additionally, it suspends the progression of ongoing inflammatory reactions. It turned out that diazepam when paired with a stimulant of immune reactions (here: lipopolysaccharide, LPS), diminished the release of pro-inflammatory molecules such as IL-12, TNF-a, and IL-6 by macrophages and dendritic cells. Furthermore, diazepam alters the immune reaction of peritoneal cells (which play a crucial role in inflammation and immune responses within the peritoneal cavity) from pro-inflammatory to anti-inflammatory, enhancing the production of IL-10. Moreover, in laboratory trials, diazepam inhibits LPS-triggered dendritic cells from stimulating detrimental immune responses (Th1 and Th17, the subsets of CD4$^+$ T helper cells, which play pivotal roles in orchestrating adaptive immune responses) that are linked to conditions like experimental autoimmune encephalomyelitis (EAE), a model for multiple sclerosis (MS). Overall, these findings suggest that diazepam can act as a potent immunomodulator, capable of controlling both innate and adaptive immune responses, whether administered preventively or therapeutically [129-131].

Well-optimized flow synthesis of **166**, depicted in Scheme **27**, was elaborated with the use of ammonium bromide/ammonium hydroxide as a source of ammonia, propylene oxide as a HCl scavenger and only two micro-reactors with high energy economy was used. The final percent yield of this transformation was 96% while the obtained product's purity was 91% (determined with UPLC). Additional crystallization from acetonitrile can yield diazepam with a purity exceeding 98%.
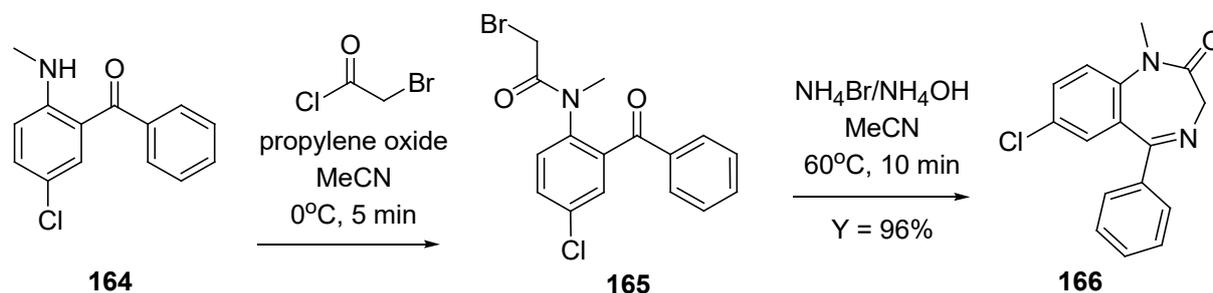
Scheme 27. Exemplary method of Diazepam synthesis (**166**).

The first stage assumes a classic nucleophilic substitution reaction, in an acyl system, between a secondary amine (**164**) and 2-bromoacetic acid chloride in the presence of propylene oxide, which captures HCl generated during this reaction, forming halogenohydrin — 1-chloropropan-2-ol as a by-product.

The second step is nucleophilic substitution between the generated ammonia and the bromoalkyl moiety in **165** followed by the intramolecular dehydrating cyclization to form a diazepin-2-one ring, resulting in the final product obtainment, **166**.

This method is characterized by reduced solvent consumption, reduced labour costs, and decreased waste production in relation to other methods. This approach can serve as a foundation for synthesizing other benzodiazepines enhancing the production and availability of essential medicines and strengthening the supply chains in the pharmaceutical industry [130].

5. Conclusions and perspectives

Numerous immunosuppressive agents have been widely applied in clinics for decades. Due to the optimization of allograft rejection prevention and serious autoimmune disorders treatment, there is still a need to improve the therapeutic properties of these drugs. As a result, novel derivatives of existing active substances and new prospective medicines on the basis of heterocyclic motifs are investigated in the scope of their molecular target, mechanism of action, and synthetic methods. In Table **1** are summarized some newly designed structures, which indicate research areas providing novel lead compound candidates, therapeutic properties improvement or promising retargeting of medicinal usage according to the known parent molecules. In fact, designed anti-proliferative agents often reveal significant antibacterial, antiprotozoal or anti-cancer potentials as well. It can be anticipated that the rapid development of active substances as chemotherapeutics will be continued.

Table 1. Newly investigated compounds as promising anti-proliferative or anti-inflammatory agents

Structure	Comment	Ref.
R - 2-Methylbenzyl, 4-Phenylbutyl **4** **5**	High *h*IMPDH2 inhibition, high activity against MDA-MB-231, DU145, U87 MG cancer cell lines.	[24]
6	Ki 0.016 μM to *Cp*IMPDH.	[34]
R- amino acid ester, **21** tuftsin moieties	MIC against *Staphylococcus aureus* 32-64 μg/ml.	[37]
76	Ki 0.76 nM to Glucocorticoid receptor, designed as inhaled corticosteroid against asthma, chronic obstructive pulmonary disease.	[81]
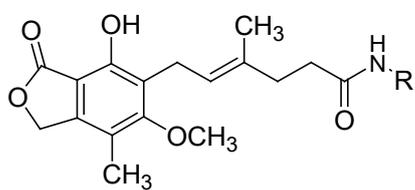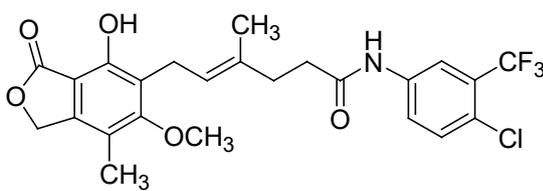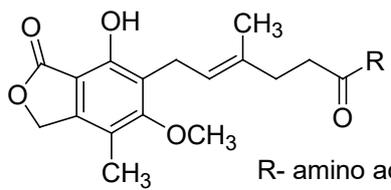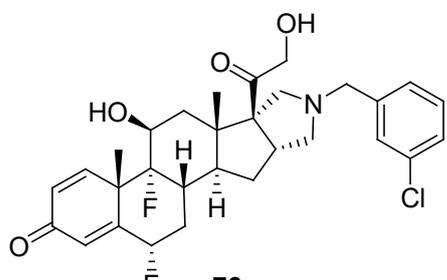
n = 1 82 **2 83**	Improved water solubility and anti-inflammatory activity. [82]
92	A safer immunosuppressant than azathioprine, with lower risks of side effects like myelosuppression and hepatotoxicity. Effective for treating steroid-resistant renal conditions. [88,89]
100	The selective inhibitor of the ATP-binding site of JAK1, crucial in regulating IL-2, IL-6, and TNF-α, along with JAK2, which influences cell proliferation and differentiation. [92,93]
105	A DMARD-class immunomodulator, exhibiting immunosuppressive, antirheumatic, antineoplastic, and antiviral properties in preclinical trials. [95]
116	A Phase 3 drug candidate reducing blood lymphocytes, improving tissue health and reducing inflammation (*in vivo*). The S1P1 receptors' internalization promoter in lymphocytes of healthy individuals and those with SLE. [98]
122	A highly potent and selective small-molecule p22phox inhibitor outperforms current peptide-mimetics. It offers advantages like transmembrane migration, cost-effectiveness, potential oral administration, and fewer immune reactions, making it a promising option for RA treatment development. [100]

45

127

A reverse inhibitor of perforin (both *in vitro* and *in vivo* conditions), tested for reducing graft rejection in bone marrow/stem cell transplantation; a good immunomodulating drug candidate.

[101]
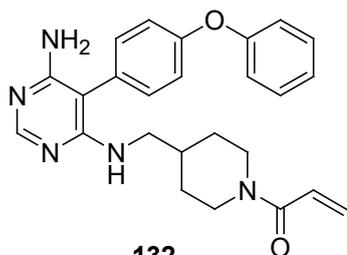
132

The BTK inhibitor, which penetrates the CNS, undergoing phase III clinical trials for MS. It has demonstrated good tolerance in RA and SLE, with no dose-related adverse events observed.

[104]
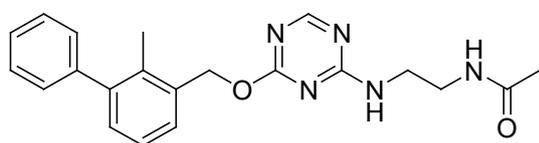
133

The inhibitor targeting the PD-1/PD-L1 system demonstrates an IC_{50} value of 0.115 μM. Its administration leads to increased secretion of IFN-γ and enhanced induction of cell death in cancer cells PC9 and HCC827.

[106]
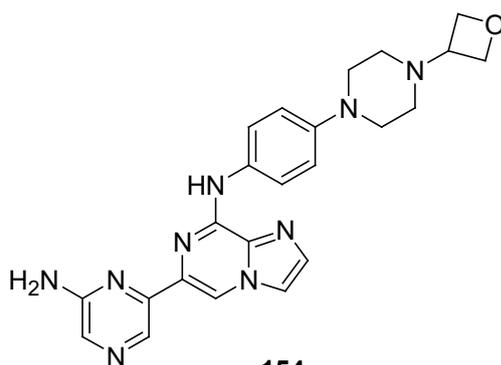
154

It effectively halted LN progression in mice, improving survival and reducing proteinuria and blood urea nitrogen levels. Results suggest it may slow disease progression in humans by targeting B cell maturation.

[112]
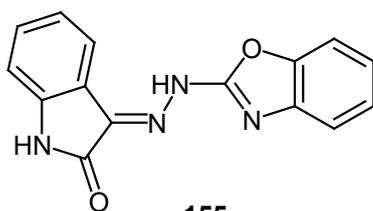
155

It targets and inhibits SGK1 activity with an IC_{50} value of 0.7 μM, what in turn results in T-cell proliferation inhibition. Preclinical studies in mice demonstrated efficacy in reducing skin inflammation caused by both dinitrofluorobenzene and imiquimod.

[113]
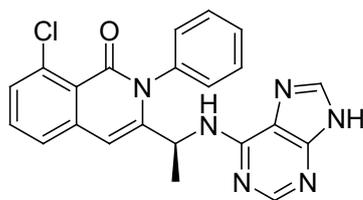
160

The FDA has positively reviewed it as a potential remedy for CLL and SLL, attributed to dysregulation of the PI3K signaling pathway.

[115]

166
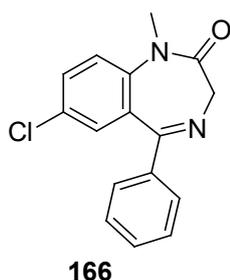
As a TSPO ligand, it functions as a potent immunomodulator, regulating both innate and adaptive immune responses. This ability enables it to dampen exaggerated immune reactions, thereby reducing inflammation and potentially providing therapeutic benefits in conditions marked by excessive immune activation, such as autoimmune diseases or septic shock.

[129-131]

Abbreviations used

Ac$_2$O — acetic anhydride

ACN — acetonitrile

AcOEt — ethyl acetate

AcOH — acetic acid

AcONa — sodium acetate

9-BBN — 9-borabicyclo[3.3.1]nonane

B$_2$pin$_2$ — bis(pinacolato)diboron, octamethyl-2,2′-bi-1,3,2-dioxaborolane

BnBr — benzyl bromide

Boc$_2$O — di-*tert*-butyl dicarbonate, Boc anhydride

BP — boiling point

CDI — 1,1'-carbonyldiimidazole

1,4-D — 1,4-dioxane

DBH — 1,3-dibromo-5,5-dimethylhydantoin

DBU — 1,8-diazabicyclo[5.4.0]undec-7-ene

DIPEA — *N*,*N*-diisopropylethylamine

DMAP — 4-(dimethylamino)pyridine

DMF — *N*,*N*-dimethylformamide

DMSO — dimethyl sulfoxide

EDCI·HCl — *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride

Et$_3$N — triethylamine

HATU — *O*-(7-aza-1*H*-benzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate

HOBt — 1-hydroxybenzotriazole

MeCN — acetonitrile

NBS — *N*-bromosuccinimide

NIS — *N*-iodosuccinimide

NMO — *N*-methylmorpholine N-oxide

O.N. — overnight

Pd(dppf)Cl$_2$ — [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II)

PPTS — pyridinium *p*-toluenesulfonate

p-TsOH — *p*-toluenesulfonic acid

Py — pyridine

[Rh(COD)Cl]$_2$ — 1,5-cyclooctadienerhodium(I) chloride dimer

RT — room temperature

SPhos — dicyclohexyl(2′,6′-dimethoxy[1,1′-biphenyl]-2-yl)phosphane

T — toluene

T<5ºC — temperature lower than 5ºC

TBDMS-Cl — *tert*-butyldimethylsilyl chloride

TBTU — 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate

TEA — triethylamine

Tf$_2$O — trifluoromethanesulfonic anhydride

TFA — trifluoroacetic acid

THF — tetrahydrofuranu, oxolane

TsCl — *p*-toluenesulfonic chloride

Y — percent yield

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Disclosure statement

The authors state that they do not have any known conflicting financial interests or personal relationships that might have appeared to exert influence on the work presented in this paper.

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