HETEROCYCLES, Vol. 96, No. 11, 2018, pp. 1869 - 1887. © 2018 The Japan Institute of Heterocyclic Chemistry
Received, 19th June, 2018, Accepted, 10th August, 2018, Published online, 19th September, 2018
DOI: 10.3987/REV-18-893

SYNTHESIS, REACTION AND BIOLOGICAL ACTIVITY OF PYRAZOLO[5,1-*c*][1,2,4]BENZOTRIAZINE 5-OXIDES

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Abstract – [1,2,4]Benzotriazine fused to pyrazole ring is a privileged heterocyclic scaffold present in numerous pharmacologically active compounds. Till date, various pyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxides and their deoxy analogues, possessing a broad spectrum of potent pharmacological activities, have been reported. In this review, we highlighted synthesis, chemical reactions, medicinal chemistry aspects and applications of fused pyrazole derivatives of [1,2,4]benzotriazine analogues reported so far.

1. INTRODUCTION

The development of 'privileged heterocyclic scaffolds' is a fast growing topic in modern medicinal chemistry. It is well documented that the pharmacological activity increases when an additional heterocyclic ring is fused to the benzotriazine nucleus.[1] Fused benzotriazines are an important class of heterocyclic compounds in medicinal chemistry due to their affinity for various biotargets. Up to date, a plenty of methods have been reported for the synthesis of potent substituted pyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxide ligands. One of the most common methods for preparation of pyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxides (**2**) involves ring closure of the intermediate aminopyrazoles (**1**) (Figure 1).

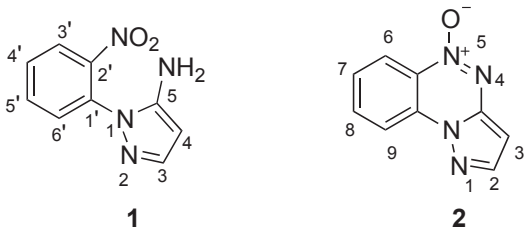


Figure 1. Numbering of aminopyrazoles (**1**) and pyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxide (**2**)

The $GABA_A$ receptor is an ionotropic receptor and ligand-gated ion channel. Its endogenous ligand is γ-aminobutyric acid (GABA), the major inhibitory neurotransmitter in the central nervous system.[2] $GABA_A$ receptors are directly associated with a Cl^- ion channel and are composed of subunits arranged as a pentameric structure (a sequence of 6α, 3β, 3γ, δ, and 2ρ subunits have been identified by molecular cloning). The heterogeneity and subunit composition determine the physiological and pharmacological properties of $GABA_A$ receptors.[3]

In the central nervous system, $GABA_A$ receptors are allosterically modulated by a number of compounds of therapeutic interest, such as the classical benzodiazepines, acting at a "benzodiazepine binding site" on $GABA_A$-receptor.[4]

Pyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxide ligands have been evaluated for their binding properties in the benzodiazepine site on $GABA_A$ receptor.[5] To investigate the binding affinity of $GABA_A$ receptor subtype, 5-deaza analogue of pyrazolo[5,1-*c*][1,2,4]benzotriazine system (**3**) *i.e.* pyrazolo[1,5-*a*]quinazolines (**4**) was chosen and *in vitro* evaluated. With disappointing, the 4- or 5-substitutions on this new system gave compounds lacking recognition of receptor protein (Figure 2).[6]

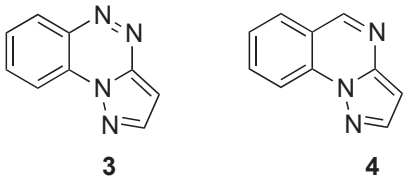


Figure 2. Pyrazolo[5,1-*c*][1,2,4]benzotriazine (**3**) and its 5-deaza analogue (**4**)

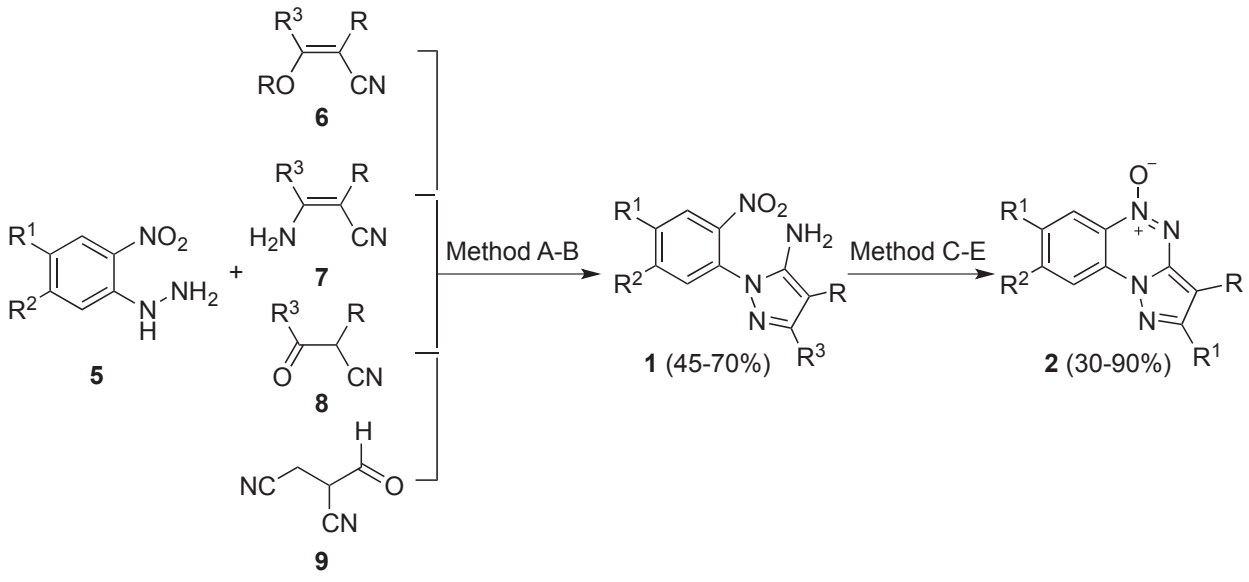
Considering the importance of this topic, here we report a summary of the chemical reactions, applications, pharmacological aspects and therapeutic effects of pyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxide derivatives reported from 1994 to date.

2. PYRAZOLO[5,1-*c*][1,2,4]BENZOTRIAZINE 5-OXIDE

2.1. Synthesis of pyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxide derivatives

Costanzo and coworkers synthesized a series of benzodiazepine receptors (BZR)

pyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxides and their 8-chloro derivatives substituted with aromatic or heteroaromatic groups. To synthesize the 2- or 3-substituted pyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxides (**2**), first the desired 5-aminopyrazoles (**1**) were obtained by two different synthetic protocols. By treating 2-nitrophenylhydrazines (**5**) with 2-cyano-3-alkoxypropenoate (**6**), 3-aminoacrylonitrile derivatives (**7**), 2-aryl-3-oxapropanenitrile (**8**), and 2-oxosuccinonitrile (**9**) corresponding 5-aminopyrazoles (**1**) were prepared.[7] Compounds (**1**) were cyclized through intramolecular cyclization to the benzotriazine systems (**2**) under basic conditions (Scheme 1).[8] The binding affinity of 3-heteroaryl-8-chloropyrazolo-[5,1-*c*][1,2,4]benzotriazine 5-oxides (**2**) at the BZR were investigated. The derivatives substituted at the 3-position with electron-rich five-membered rings such as 2-thiophene or 3-thiophene, showed good affinity values for BZR. In *in vivo* tests, the 3-(thien-3-yl)-8-chloropyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxide (**2**) showed selective anticonvulsant activity. The other compounds showed no significant affinity for BZR.[9]



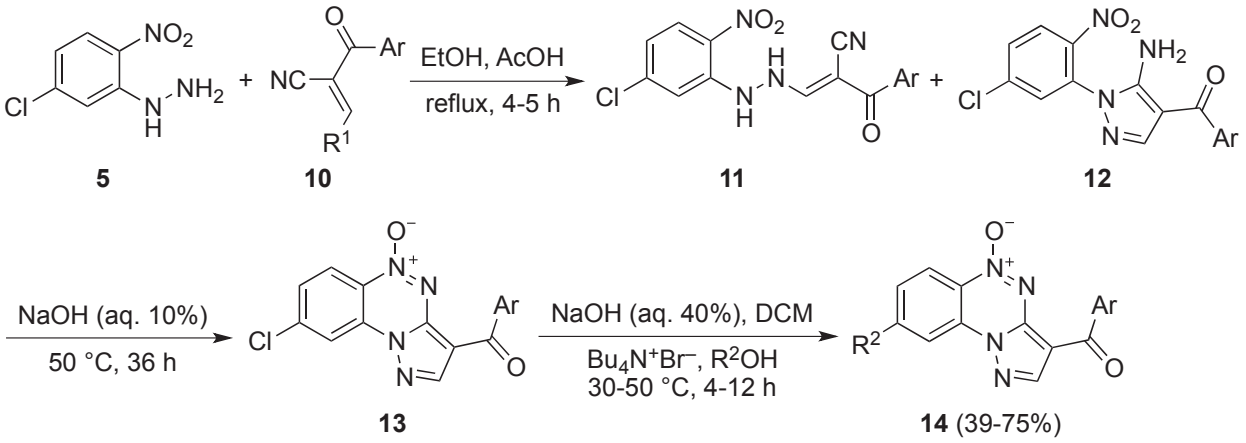
Method A: AcOH, EtOH, reflux, 12 h (48-70%)
Method B: HCl (conc.), EtOH, 60 h (45-65%)
Method C: NaOH (aq, 10%), diglyme, rt, 12-24 h (53-90%)
Method D: NaOH (aq, 40%), EtOH, reflux, 24 h, (45-65%)
Method E: 1) NaOH (aq, 10%), EtOH, rt, 24 h, 2) HCl (aq, 6 N), rt, 2-3 h (30-80%)

R= H, CN, CH_2CN, CO_2Et, CO_2H, $CONH_2$, Ph, 2-FC_6H_4, 3-FC_6H_4, 4-FC_6H_4, 2-ClC_6H_4, 3-ClC_6H_4, 4-ClC_6H_4, 3,4-$Cl_2C_6H_3$, 2-Py, 2-thienyl, 3-thienyl
R^1= H, Cl, NO_2
R^2= H, Me, OH, OEt, Cl, Br, I
R^3= H, Me, CO_2Et, Ph, 2-$MeOC_6H_4$, 2-thienyl, 3-thienyl, 2-furyl

Scheme 1. Cyclization of 1-(2-nitro-4,5-disubstituted-phenyl)-5-aminopyrazoles (**1**)

In another study, from the reaction of 5-chloro-2-nitrophenylhydrazine (**5**) and 2-ethoxymethylene-3-oxobutanenitrile or 2-(dimethylaminomethylene)-3-oxo-3-arylpropanenitrile (**10**) in the presence of

acetic acid as catalyst, hydrazinyl derivative (**11**) and 3-acylpyrazolobenzotriazines (**12**) were obtained. 3-Acyl-8-chloro[5,1-*c*][1,2,4]benzotriazine 5-oxide systems (**13**) were obtained by ring closure of compounds (**12**) in alkaline medium. Finally, 3-alkylcarbonyl, 3-acyl-8-alkoxy- or 8-aryloxypyrazolo-[5,1-*c*][1,2,4]benzotriazine 5-oxide (**14**) were achieved through exploiting the phase transfer catalyzed (PTC) chlorine displacement at position 8 of the benzotriazine system. In this procedure, a suitable reagent (phenol, benzyl alcohol, or propargyl alcohol) was added to a two-phase system consisting of a strong aqueous sodium hydroxide solution, a catalyst tetrabutylammonium bromide ($Bu_4N^+Br^-$), and a methylene chloride solution of starting materials (Scheme 2). Among the synthesized derivatives, 3-theonyl-8-phenoxypyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxide showed a high binding affinity value for BZR and selective anxiolytic activity devoid of side effects.[10]



5 **10** **11** **12**

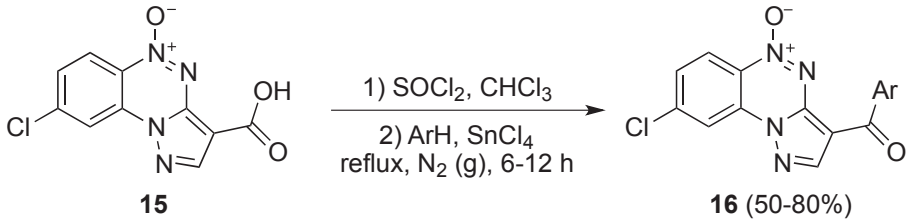
13 **14** (39-75%)

Ar= Ph, 2-$MeOC_6H_4$, 4-$MeOC_6H_4$, 2-furyl, 2-thienyl, 3-thienyl, 2-pyrrolyl, 3-Py, 1,3-benzodioxol-5-yl
R^1= OEt, NMe_2
R^2= OEt, OPh, OBn, *O*-propargyl

Scheme 2. Synthetic route for 3-aroyl-8-chloropyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxides (**14**)

2.2. Reactions of 3,8-disubstituted pyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxide derivatives

Guerrini and coworkers utilized two synthetic approaches to obtain the desired 3-acyl-8-chloropyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxides (**16**). Firstly, by treatment of 3-carboxy-8-chloropyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxide (**15**) with thionyl chloride the corresponding 3-acyl chloride was obtained as an intermediate. Then, this intermediate was directly converted into final product (**16**) using the Friedel-Crafts reaction (Scheme 3). When the substituent of the carbonyl group was a five membered heteroaroyl group, the affinity strongly increased with a K_i value in the sub-nanomolar range. Probably heteroaroyl derivatives (3-(2-furoyl)- and (3-(2-thenoyl)- interact with receptor protein in a different way with respect to 3-(pyrrole-2-carbonyl) derivative.[10]

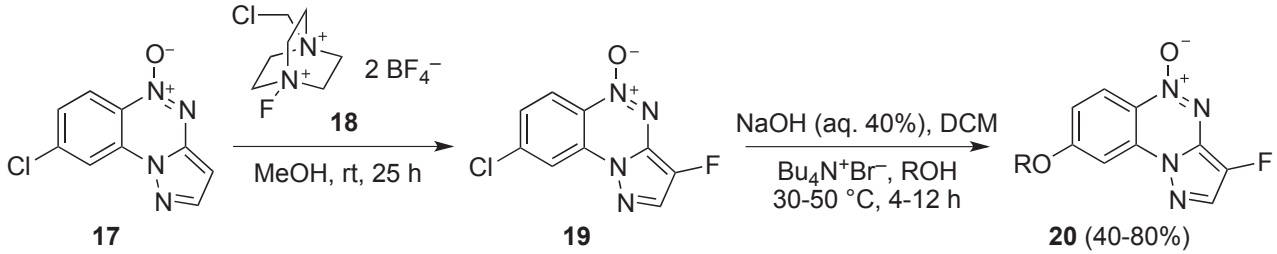


15 **16** (50-80%)

Ar= Ph, 4-$MeOC_6H_4$, 2-$MeOC_6H_4$, 3-Py, 2-furyl, 2-thienyl, 3-thienyl, 2-pyrrolyl, 1-Me-2-pyrrolyl, 1-Me-3-pyrrolyl, 1,3-benzodioxol-5-yl

Scheme 3. Synthesis of 3-aroyl-8-chloropyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxides (**16**)

Guerrini's research group described the synthesis of 3-fluoro-8-alkyloxy-/arylmethoxypyrazolo-[5,1-*c*][1,2,4]benzotriazine 5-oxides (**20**). In this regard, 3-fluoro-8-chloropyrazolo[5,1-*c*][1,2,4]-benzotriazine 5-oxide (**19**) was obtained from the reaction of the corresponding 3-unsubstituted compound (**17**) with a fluorinating agent, 1-chloromethyl-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane bis(tetrafluoroborate) (F-TEDA-BF_4) (**18**). Finally, 3-fluoro-8-alkyloxy/arylmethoxy derivatives (**20**) were achieved by the nucleophilic substitution of the chlorine atom at position 8 of the compound (**19**) under PTC conditions (Scheme 4). An analysis of all synthesized compounds confirmed the essential interaction points for binding recognition and the important areas for affinity modulation. The fluorine atom was able to form a hydrogen bond interaction.[11]



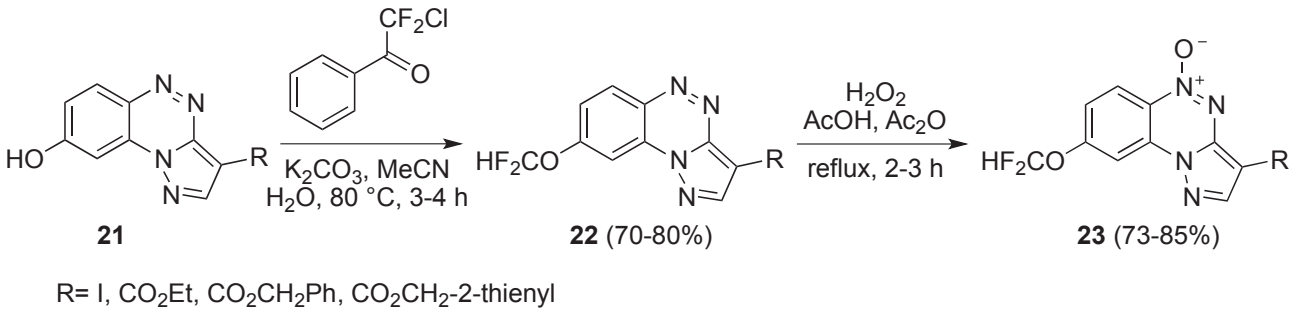
17 **19** **20** (40-80%)

R= propargyl, 2-$MeOC_6H_4CH_2$, 2-$CF_3C_6H_4CH_2$, 4-Py-CH_2, 2-thienyl-CH_2, 2-furyl-CH_2

Scheme 4. Preparation of 3-fluoro-8-alkyloxy-/arylmethoxypyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxides (**20**)

Investigations by Guerrini and coworkers showed that the introduction of difluoromethoxy functionality is applicable by exploiting the 8-hydroxy group on the pyrazolobenzotriazine system. In this regard, the reaction with a new difluorocarbene reagent, 2-chloro-2,2-difluoroacetophenone, was made on the 5-deoxide derivatives (**21**). Then, 3-iodo-, 3-ethoxycarbonyl-, 3-benzyloxycarbonyl and 3-(2-thienylmethoxycarbonyl)-8-difluoromethoxypyrazolo[5,1-*c*][1,2,4]benzotriazines (**22**) were oxidized with acetic anhydride/hydrogen peroxide to achieve the corresponding 5-oxide derivatives (**23**) (Scheme 5). The binding properties of the fluorine atom(s) in the Bz site on $GABA_A$ receptors and their ability to render

compounds more metabolically stable were evaluated. The findings indicate that the fluorine atom is very important for recognition, engaging hydrogen bond intraction, when it is in position 8 interested in the 8-difluoromethoxy groups.[11]



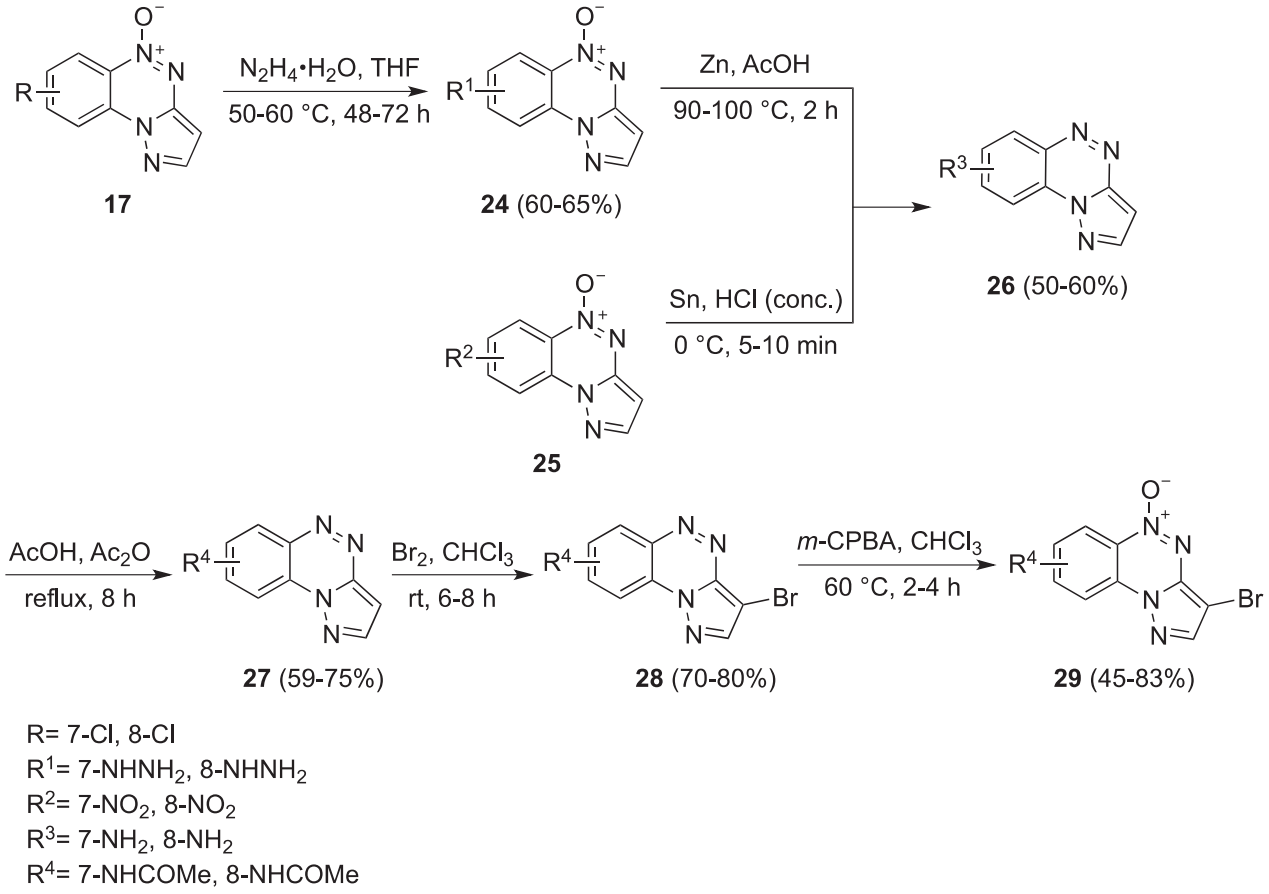
Scheme 5. Synthesis of 3-substituted-8-difluoromethoxypyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxides (**23**)

The reaction of 7-chloro and 8-chloropyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxides (**17**) with hydrazine hydrate afforded the 7- and 8-hydrazinyl derivative (**24**). Compounds (**24**) by reduction with zinc/acetic acid yielded the expected 8-aminopyrazolo[5,1-*c*][1,2,4]benzotriazines (**26**). On the other hand, the reaction of nitropyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxides (**25**) with tin/conc. hydrochloric acid furnished compounds (**26**) as the only products. Acetylation of the amino group in compounds (**26**) and the subsequent bromination of the 3-position, resulted in the formation of 7- and 8-acetylamino-3-bromo derivatives (**28**). Finally, upon oxidation of these compounds (**28**) with *m*-CPBA, the desired 5-oxide derivatives (**29**) were obtained (Scheme 6). The BZR affinity of the 5-oxides was better than reduced derivatives and did not correspond with the best activity in *in vivo* tests, where the anticonvulsant effects of the two series were comparable.[12]

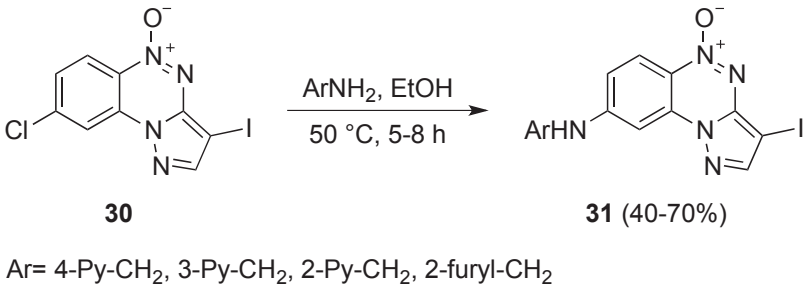
New 3-iodo-8-heteroarylmethylamino derivatives (**31**) were synthesized from 3-iodo-8-chloropyrazolo-[5,1-*c*][1,2,4]benzotriazine 5-oxide (**30**) by addition of an excess of suitable heteroarylamines in the presence of ethanol (Scheme 7).[13]

In another study, 3-carboxy-8-chloropyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxide (**15**) was used as starting material to obtain 3-methyl-8-chloropyrazolo[5,1-*c*][1,2,4]benzotriazine (**32**) using borane dimethyl sulfide complex (BMS) as reducing agent. This borane complex was reported to reduce the carboxy group to methyl group. In the second step, compound (**32**) was oxidized to the corresponding *N*-5-oxide (**33**). Finally, compounds (**34**) were prepared by nucleophilic substitution at position 8 of pyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxide (**33**). The chlorine displacement occurred by PTC. In this procedure, the suitable alcohol was added to a two-phase system consisting of a strong aqueous sodium hydroxide solution, tetrabutylammonium bromide as catalyst, ($Bu_4N^+Br^-$), and a methylene chloride

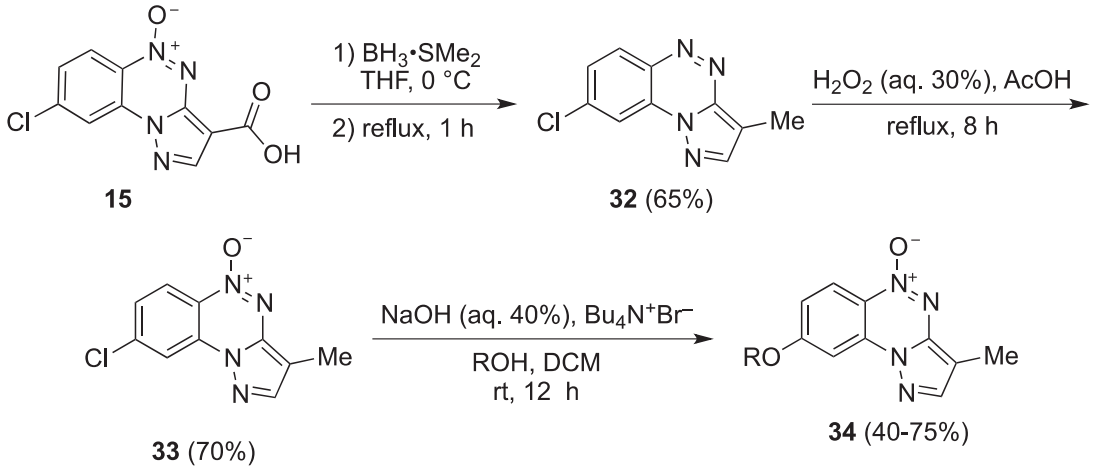
solution of starting material (Scheme 8). *In vitro* and *in vivo* tests have been performed. Pharmacological data indicated that compounds (**34**) stand out as selectively displaying good antiamnesic and procognitive activities.[13] The 8-O-CH_2-4-pyridyl chain of compound (**34**) standed out as the compound that improved mouse memory processes selectively, safely, and in a statistically significant manner.[14]



Scheme 6. Synthesis of pyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxides (**29**) with different substituents at the 7- or 8-position



Scheme 7. The synthesis of 3-iodo-8-heteroarylmethylamino derivatives (**31**)

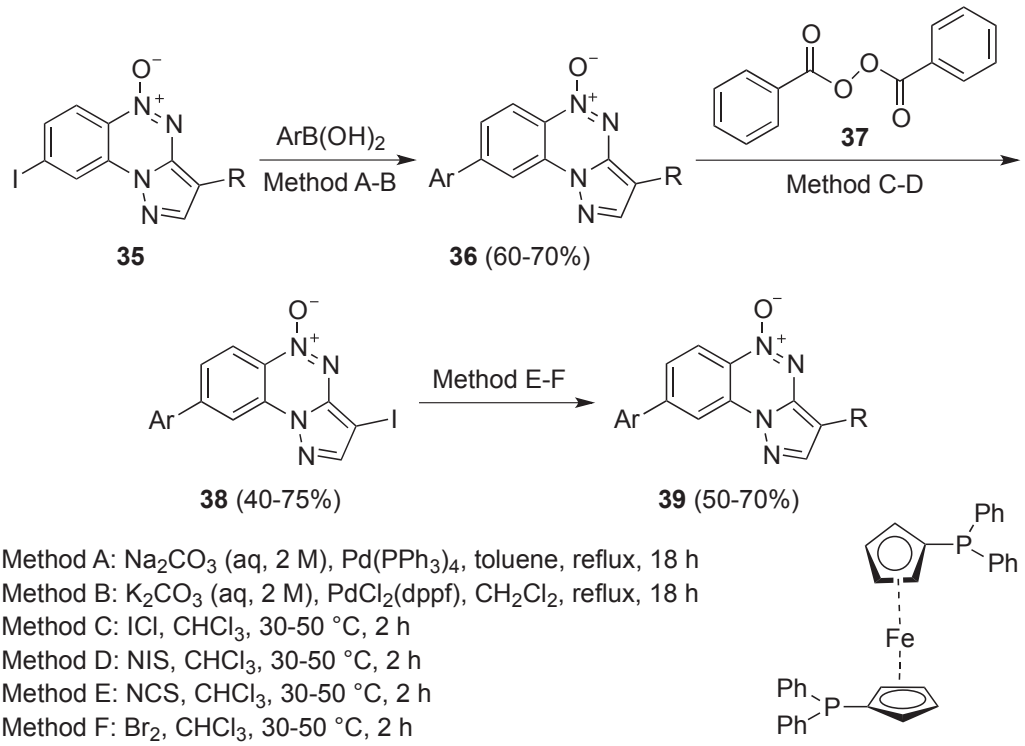


R= H, Me, Et, *i*-Pr, Pr, *n*-Bu, *n*-Pent, CH_2-cyclopropyl, $(CH_2)_2OMe$, $(CH_2)_2NMe_2$, $(CH_2)_2Cl$, propargyl, *n*-Oc, Cy, Ph, Bn, 2-naphthyl, CH_2-2-$MeOC_6H_4$, CH_2-4-$MeOC_6H_4$, CH_2-2-$OCF_3C_6H_4$, CH_2-4-ClC_6H_4, *i*-PrC_6H_4, *i*-PrC_6H_4, *i*-PrC_6H_4, CH_2-4-Py, CH_2-2-furyl, $(CH_2)_2C_6H_4$, *i*-BuC_6H_4, *sec*-BuC_6H_4

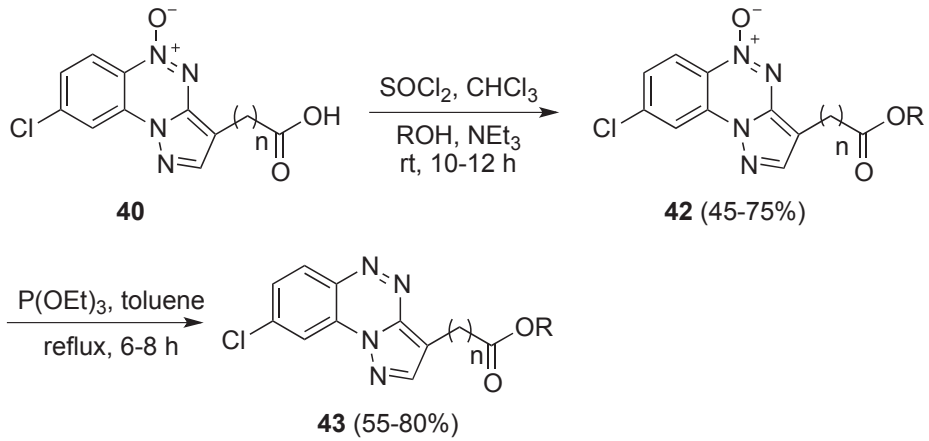
Scheme 8. Synthesis of 3-substituted-8-alkoxypyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxides (**34**)

8-Aryl derivatives of pyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxides (**36**) have been obtained through the reaction of 3-halo-8-iodopyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxides (**35**) with suitable boronic acids, using $Pd(PPh_3)_4$ or [1,1'-bis(diphenylphosphino)ferrocene]palladium(II) chloride $PdCl_2$(dppf) as catalysts. Halogenation by iodine monochloride in chloroform in position 3 of the pyrazole ring was the second step to obtain the desired products 3-iodo-8-aryl or alkylpyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxide derivatives (**38**). Finally, by treating compounds (**38**) with halogenating reagents, *N*-chlorosuccinimide (NCS) or bromine, the corresponding 3-chloro- and 3-bromo- derivatives were achieved (**39**) (Scheme 9).[14]

Costanzo's research group synthesized the 3-ester derivatives (**43**) by addition of thionyl chloride and suitable alcohol to 3-carboxy-8-chloropyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxide or 3-carboxymethyl-8-chloropyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxide (**40**) in 2-methyl-3-butene stabilized chloroform solvent to prepare compounds (**42**). Then, compounds (**42a-z**) were treated with triethylphosphite in toluene solvent (Scheme 10). 3-(2-Methoxybenzyloxycarbonyl)-8-chloropyrazolo[5,1-*c*][1,2,4]-benzotriazine 5-oxide (**41**) showed an excellent affinity value in binding test. Among the heteroaryl esters, 3-(2-thienylmethoxycarbonyl)-8-chloropyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxide (**42**) that exhibited good binding affinity was found to be a selective ligand *in vivo*. Consequently, it showed anxiolytic-like activity in the conflict models (light-dark box and plus maze test) similar to diazepam.[15]



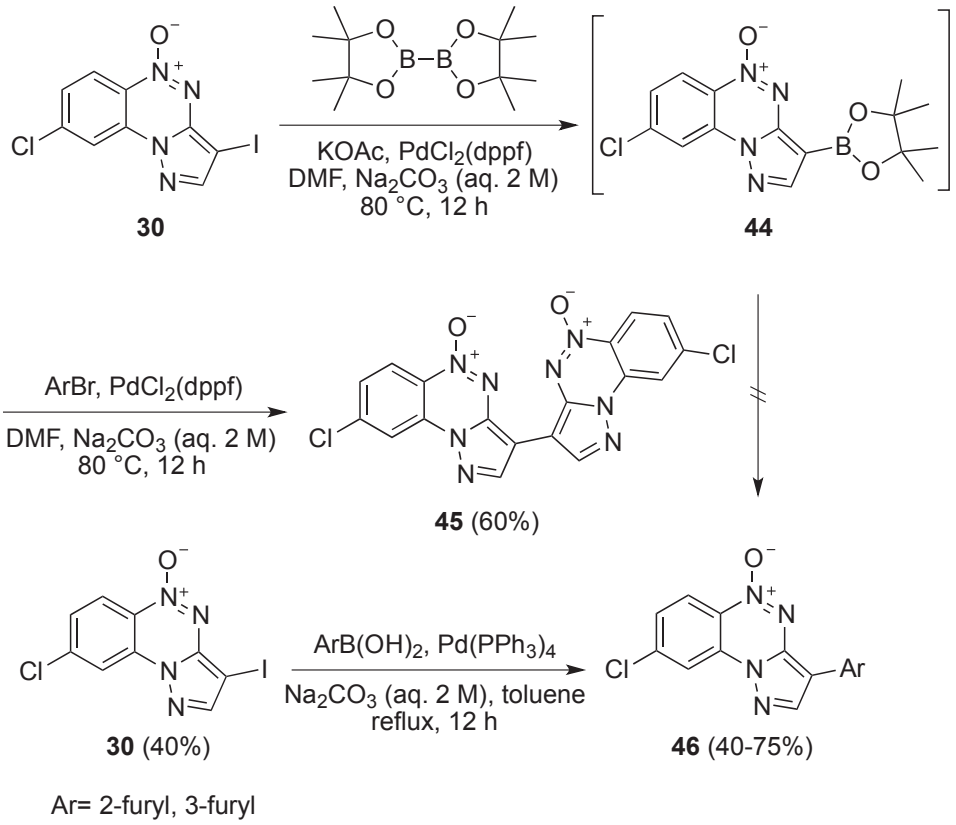
Scheme 9. Synthesis of 3-halo-8-arylpyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxides (**39**)



Scheme 10. Synthetic path of 3-esters of the 8-chloropyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxides (**42**)

Guerrini and coworkers attempted to synthesize 3-(3-furyl)-8-chloropyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxide (**46**) from 3-iodo-8-chloropyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxide (**30**) in the presence of 2- or 3-furanboronic acid. Since 2- or 3-furanboronic acids were not commercially available, an alternative synthetic route was investigated. They found that palladium-catalyzed coupling reactions of

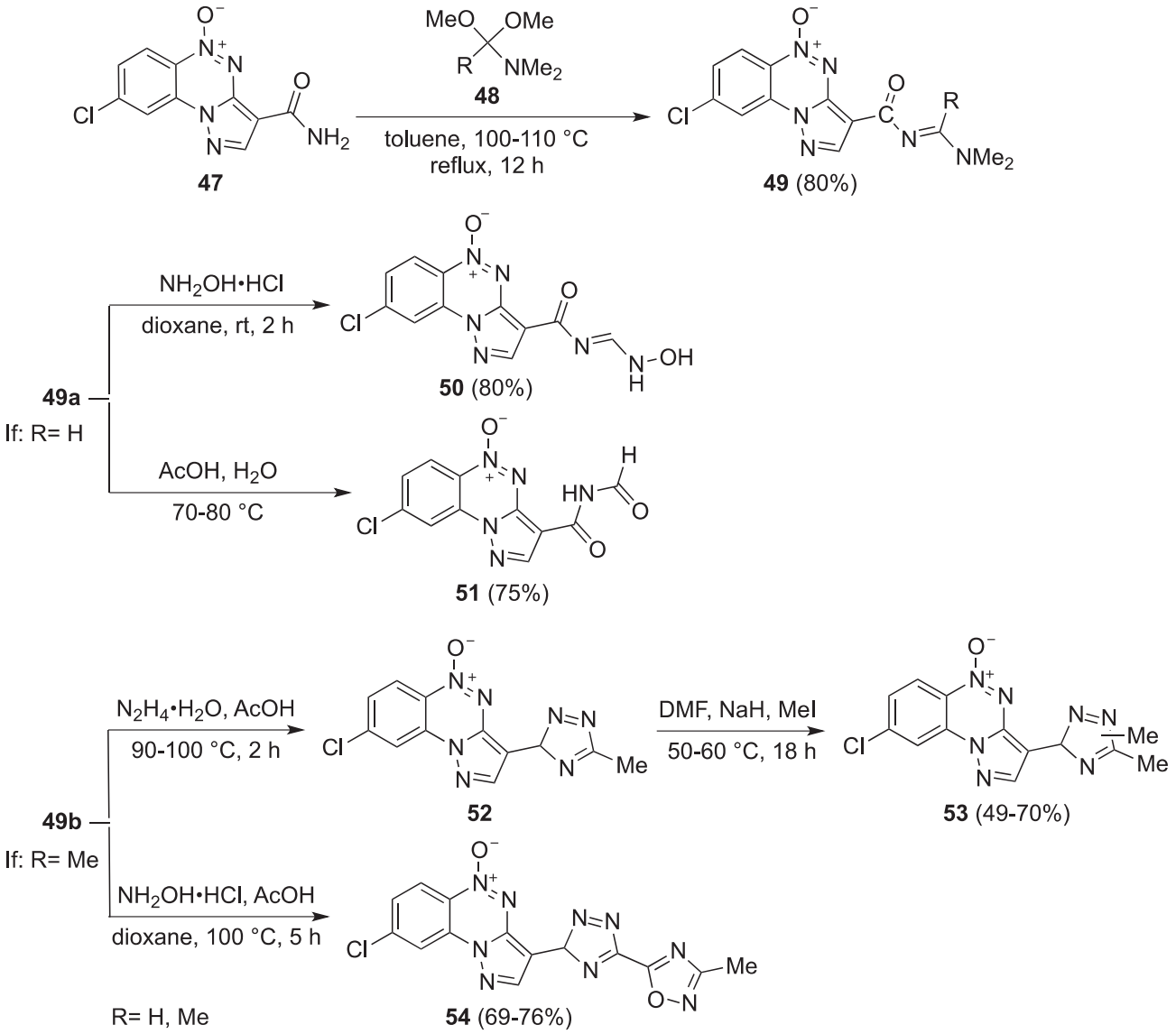
bis(pinacolato)diboron and aryl halides, could be useful synthetic tools for preparing unsymmetrical biaryls. By using 3-iodo-8-chloropyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxide (**30**) as aryl halide to obtain arylboronic ester intermediates (**44**), to which 3-bromo- and 2-bromofuran had to be added, always dimer (**45**) was obtained as the main compound and the expected compound isomeric 3-(3-furyl) and 3-(2-furyl)-8-chloropyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxide (**46**) was not formed (Scheme 11). The binding affinities at the BZR for the 3-(2-furyl)- and 3-(3-furyl)-8-chloropyrazolo[5,1-*c*][1,2,4]-benzotriazines 5-oxides (**46**) indicated a preferential affinity at different receptor subtypes. The binding affinities at the BZR and the muscle relaxant, anticonvulsant and anxiolytic activities of new furyl derivatives were compared to those of the 3-heteroaryl derivatives BZR ligands previously reported. The results confirmed the stringent spatial requisites of the lipophilic pocket that accommodate the 3-substituent, which seems to influence both the binding and intrinsic activity of this class of ligands. Furthermore, compounds (**46**) indicated preferential affinities at different receptor subtypes.[16]



Scheme 11. Synthesis of 3-(2-furyl)- and 3-(3-furyl)-8-chloropyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxides (**46**)

Costanzo and coworkers synthesized the acyl amidines (**49**) from the reaction of 3-carbamoyl-8-chloropyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxide (**47**) with dimethylformamide dimethyl acetal (DMF-DMA) or dimethylacetamide dimethyl acetal (DMA-DMA) (**48**). Next, compound (**49a**) in

the presence of hydroxylamine hydrochloride gave only the corresponding 3-*N*-hydroxyaminomethylene amide (**50**). Upon hydrolysis of acyl amidine group of compound (**49a**) through H_2O/AcOH, 3-*N*-formylamide derivative (**51**) was also obtained. On the other hand, the compound (**49b**) was cyclized with hydrazine hydrate to obtain 3-([1,2,4]triazol-3-yl) derivatives (**52**). Then, compounds (**52**) were alkylated by methyl iodide/dimethylformamide on *N*-1,2 and 4 atom to yield the methyl derivatives (**53**). Finally, the same intermediate acylamidine (**49b**) was applied to achieve the 3-([1,2,4]oxadiazol-5-yl) derivatives (**54**) by treatment with hydroxylamine hydrochloride in acetic acid. (Scheme 12).[17]

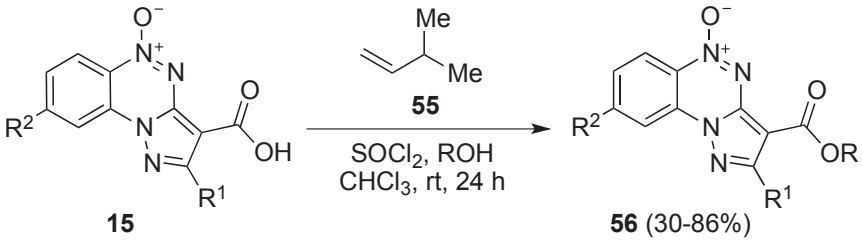


Scheme 12. Synthetic path of 3-heteroaryl-8-chloropyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxides (**50-54**)

2.3. Reactions of 2,3,8-trisubstituted pyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxide derivatives

Costanzo's research group prepared 3-ester derivatives (**56**) from appropriate 3-carboxypyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxide (**15**) by treatment with thionyl chloride followed by

addition of a suitable alcohol in 3-methyl-1-butene (**55**) stabilized chloroform (Scheme 13).[18] The binding affinities at the BZR of pyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxide derivatives (**56**) were evaluated. 3-Propynyl ester and 3-benzyl ester had the best affinity values. When the ester group was transformed into thioloester group, the BZR affinity was maintained even if less than corresponding oxyesters. Therefore, the isosteric replacement of the other oxygen by a sulfur atom in the ester function, did not improve the affinity of new compounds, even if on the aromatic ring there was the most suitable substituent as methoxy group.[19]



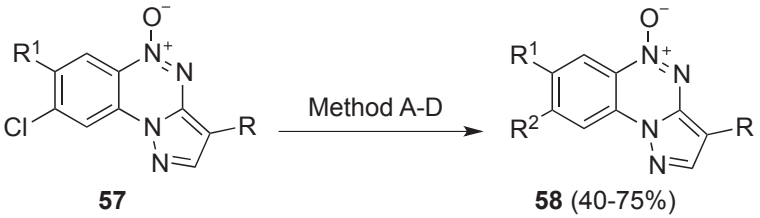
R= Me, Et, Pr, Bu, *t*-Bu, $(CH_2)_2Cl$, $(CH_2)_2Br$, $(CH_2)_2OMe$, $(CH_2)_2NEt_2$, propargyl, cyclohexyl, CH_2cyclopropyl, $(CH_2)_2$cyclohexyl, Ph, Bn, $(CH_2)_2Ph$, CH_2-2-naphthyl, $(CH_2)_2Cl$
R^1= H, Me
R^2= H, F, Cl

Scheme 13. Synthesis of 3-alkyloxycarbonyl- or 3-aryloxycarbonyl derivatives of pyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxides (**56**)

2.4. Reactions of 3,7,8-trisubstituted pyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxide derivatives

It has been found that nucleophilic aromatic substitution of 8-chloropyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxides (**57**) with nucleophilic reagents under different conditions (*N*-methylpiperazine, morpholine, dimethylethanolamine, and pentanol) with chlorine atom at position 8 resulted in the formation of compounds (**58**) (Scheme 14). 3-Nitro-7-methyl-, 3-nitro-7-trifluoromethyl- and 7-nitro-8-*N*-methylpiperizinepyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxides (**58**) displayed IC_{50} values on the growth of the four human cancer cell lines (HT29 and HCT-8, colon carcinoma, MCF7, breast carcinoma, and A549, lung carcinoma cells).[20]

A series of 3-ester derivatives of 7,8-disubstituted pyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxides (**60**) were obtained from corresponding acids (**59**) by treatment with triethylamine/ethyl chlorocarbonate in tetrahydrofuran to achieve the not isolated, mixed anhydride, which was reacted with suitable alcohol (Scheme 15). The compound 3-(2-methoxybenzyloxycarbonyl)- and 3-(2-thienylmethyloxycarbonyl)-8-trifluoromethylpyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxide (**60**) predominated because of their selective anxiolytic-like profile without side effect. The compounds (**60**) had inverse-agonist profile because they showed anxiogenic and promnemonic activity.[21]

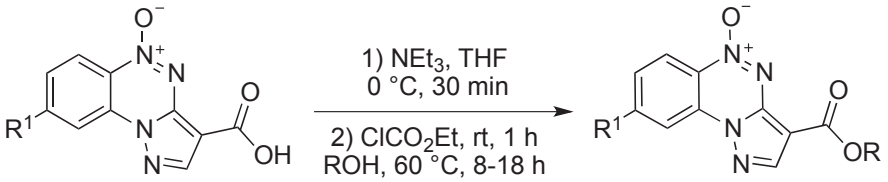


57 **58** (40-75%)

R= H, Ph, NO_2
R^1= Me, CF_3, NO_2
R^2= *N*-methylpiperazine, morpholine, $O(CH_2)_2NMe_2$, $O(CH_2)_4Me$

Method A: *N*-methylpiperazine, $EtO(CH_2)_2OH$, 60-100 °C, 2-12 h
Method B: morpholine, $EtO(CH_2)_2OH$, 60-100 °C, 2-12 h
Method C: $HO(CH_2)_2NMe_2$, NaOH (aq, 40%), 60-100 °C, 2-12 h
Method D: $HO(CH_2)_4Me$, NaOH (aq, 40%), 60-100 °C, 2-12 h

Scheme 14. Reaction of 3,7-disubstituted-8-chloropyrazolo[5,1-*c*][1,2,4]benzotriazine (**58**) with different nucleophilic reagents

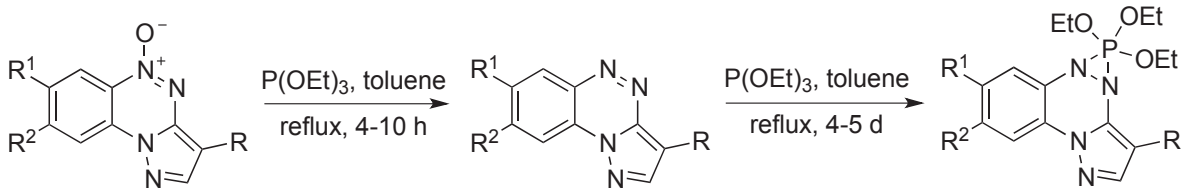


59 **60** (35-98%)

R^1= Me, OMe, OEt, SMe, CF_3, Br, I
R= CH_2-2-$NO_2C_6H_4$, CH_2-2-furyl, CH_2-2-thienyl, CH_2-3-thienyl, CH_2-2-$MeOC_6H_4$

Scheme 15. The synthetic path to 3-ester derivatives of 7,8-disubstituted pyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxides (**60**)

Costanzo and coworkers synthesized the deoxy derivatives (**62**) through the reaction of the corresponding 5-oxides (**61**) with triethylphosphite to selectively reduce the *N*-oxide group. Then, tetraazaphosphate derivatives (**63**) were obtained through heating compounds (**62**) in triethylphosphite for several days (Scheme 16). Various 3-, 7-, 8-substituted pyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxides (**61**) and their 5-deoxy derivatives (**62**) were prepared to investigate their antimicrobial, anti HIV-1 and antiproliferative activities. Consequently, derivatives bearing electron-withdrawing groups at the 3-, 7- and 8-positions showed antiproliferative activity against human neoplastic cell lines. Tested compounds prevented the proliferation of lymphoblastoid, leukemia and solid tumor cell lines. Furthermore, several compounds displayed antibacterial and antifungal activities, although at lower concentrations than those cytotoxic for lymphoblastoid cells. Both compounds (**61**) and (**62**) showed inhibitory activity against gram positive bacteria *Staphylococcus aureus*, *Streptococcus* and *M. smegmatis*. Interestingly, the tested compounds were also active against yeasts and molds and showed the highest potency against *Cryptococcus neoformans*.[22]



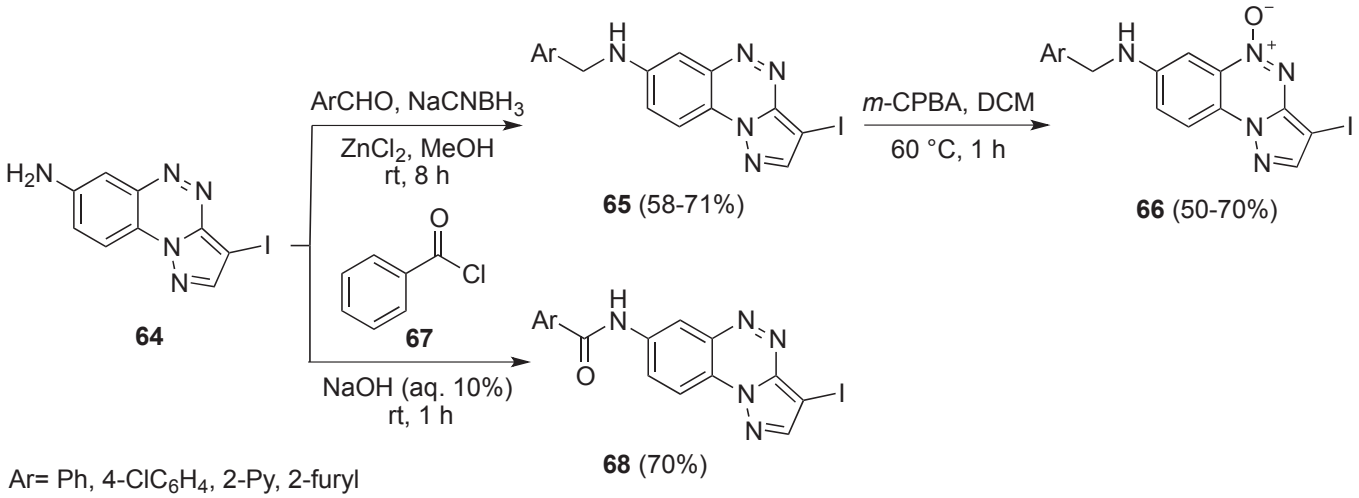
R= H, CO_2Me, CO_2Et, Ph, 4-FC_6H_4, Br, CN, NO_2
R^1= Cl, NO_2
R^2= H, Cl

Scheme 16. Preparation of pyrazolo[5,1-*c*][1,2,4]benzotriazines (**62**)

3. PYRAZOLO[5,1-*c*][1,2,4]BENZOTRIAZINE

3.1. Reactions of 3,7-disubstituted pyrazolo[5,1-*c*][1,2,4]benzotriazine

The reductive *N*-alkylation of 3-methyl-7-aminopyrazolo[5,1-*c*][1,2,4]pyrazolobenzotriazine (**64**) with suitable (hetero)arylaldehyde and sodium cyanoborohydride/$ZnCl_2$ afforded the corresponding 3-iodo-7-aryl(alkyl)amino derivatives (**65**). Upon treatment of **65** with benzoyl chloride (**67**), the corresponding *N*-(3-iodopyrazolo[5,1-*c*][1,2,4]benzotriazin-7-yl)benzamides (**68**) were obtained. Furthermore, compounds (**65**) were, in turn, oxidized with *m*-chloroperbenzoic acid (*m*-CPBA) to give the *N*-5-oxide (**66**) (Scheme 17). The synthesized compounds were tested for their affinity at $GABA_A$-receptor subtype; the compounds (**66**) were further investigated in animal models of anxiety and persistent pain. All 7-arylalkylamino derivatives, designed as isomers of the active 8-arylalkylaminopyrazolo-[5,1-*c*][1,2,4]benzotriazines, surprisingly had very low affinity at $GABA_A$-R subtype. Within this series, only 7-benzylamino derivatives (**66**) had affinity in micromolar range, showing that the phenyl of the benzylamino chain must be unsubstituted and it cannot be replaced with a heteroaryl ring.[23]

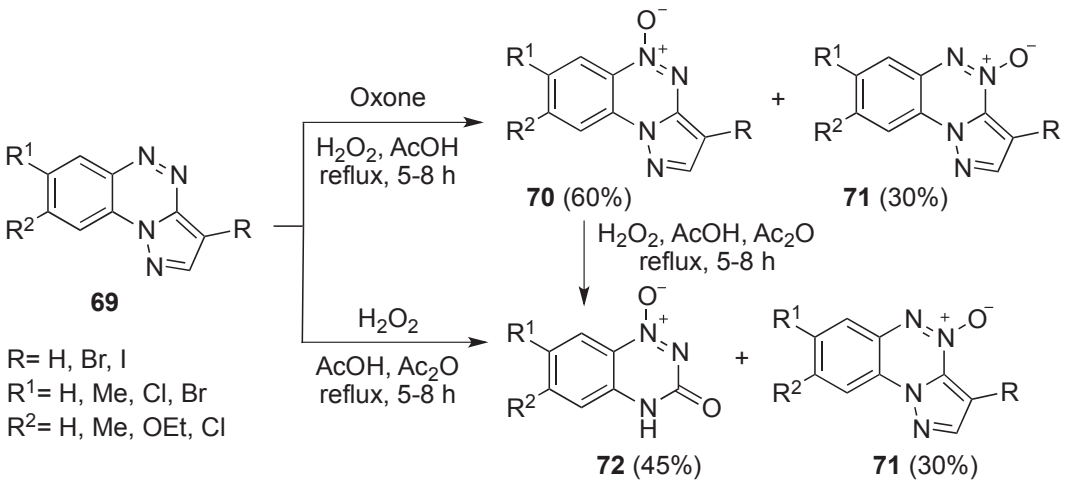


Ar= Ph, 4-ClC_6H_4, 2-Py, 2-furyl

Scheme 17. Reaction of 7-amino-3-iodopyrazolo[5,1-*c*][1,2,4]benzotriazine (**64**)

3.2. Reactions of 3,7,8-trisubstituted pyrazolo[5,1-*c*][1,2,4]benzotriazine

Costanzo and coworkers showed that oxidation of pyrazolo[5,1-*c*][1,2,4]benzotriazines (**69**) with oxone, hydrogen peroxide/acetic acid resulted in a mixture of 4-oxide (**71**) and 5-oxide compounds (**70**). On the other hand, compounds (**69**) were treated with oxidizing agent of hydrogen peroxide/acetic acid and acetic anhydride to afford 4-oxide (**71**) and 3-oxo-3,4-dihydro[1,2,4]benzotriazine 1-oxides (**72**). Fortunately, under these conditions, the 4-oxides isomers (**71**) were stable, while the pyrazole ring of 5-oxide isomers (**70**) decomposed to furnish 3-oxo-3,4-dihydro[1,2,4]benzotriazine 1-oxides (**72**) (Scheme 18). It was found that the displacement of the *N*-oxide group from the 5 position to the BZR affinities of 3,8-disubstituted-pyrazolo[5,1-*c*][1,2,4]benzotriazine 4-oxides for example 8-chloro- and 8-ethoxy-3-bromo-pyrazolo[5,1-*c*][1,2,4]benzotriazine 4-oxides (**71**) exhibited a better receptor affinity than their corresponding 5-oxide isomers (**70**) with an efficacy trend of antagonist/partial inverse agonist. Furthermore, 3-bromo-8-ethoxypyrazolo[5,1-*c*][1,2,4]benzotriazine 4-oxide (**71**) having the best affinity *in vitro* showed rather higher anticonvulsant activity in comparison with other compounds.[24] 3-Iodo-8-ethoxypyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxides (**70**) give high affinity ligands for *in vivo* testing.[25]



Scheme 18. Preparation of pyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxides (**70**) and 4-oxides (**71**)

4. CONCLUSION

In the present review, a survey of synthetic routes, chemical reactivity and biological activity aspects of pyrazolo[5,1-*c*][1,2,4]benzotriazines has been discussed. In fact, the synthesis of pyrazolo[5,1-*c*][1,2,4]benzotriazine scaffolds was closely examined from the precursor of 2-nitrophenylhydrazine with different reagents such as ethyl 2-cyano-3-ethoxypropenoate, 2-oxosuccinonitrile, 2-aryl-3-oxapropanenitrile, 2-ethoxymethylene-3-oxobutanenitrile or 2-dimethylaminomethylene-3-oxo-3-arylpropanenitrile as well as *via* the intermediate of

2-nitrophenyl-5-aminopyrazole derivatives under basic conditions. In addition, the reactions of these benzotriazine systems with different reagents were presented. For example, various oxidizing and reducing agents including oxone, hydrogen peroxide/acetic acid and tin/conc. hydrochloric acid were used to achieve various derivatives of the target compounds. The nucleophilic aromatic substitution of pyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxides with nucleophilic reagents under different conditions resulted in optimized analogues. From the pharmacological point of view, the binding affinity at the BZR of 3,8-disubstituted pyrazolo[5,1-*c*][1,2,4]benzotriazine 5-oxides were evaluated *in vitro* which indicated preferential affinity in the nanomolar range. Interestingly, those compounds that had best affinity values for BZR *in vitro* showed very weak intrinsic efficacy *in vivo* and some of them exhibited potent activity *in vivo* in comparison with other compounds. Also, some analogues have exhibited promising therapeutic effects such as antitumor, antimicrobial, anti-fungal, anticonvulsant, analgesic, antianxiety, and antihypertensive activities.

ABBREVIATIONS

BMS: Borane dimetyl sulfide

BZR: Benzodiazepine receptor

DCM: Dichloromethane

Diglyme: Diethylene glycol dimethyl ether

DMF: Dimethylformamide

DMA-DMA: Dimethylacetamide dimethyl acetal

DPPF: 1,1'-Bis(diphenylphosphino)ferrocene

F-TEDA: 1-Chloromethyl-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane bis(tetrafluoroborate)

GABA: Gamma-aminobutyric acid

$GABA_A$: Gamma-aminobutyric acid receptor, subtype A

IC_{50}: The half maximal inhibitory concentration

NCS: *N*-Chlorosuccinimide

NIS: *N*-Iodosuccinimide

m-CPBA: *meta*-Chloroperbenzoic acid

PTC: Phase transfer catalyzed

PBT: Pyrazolobenzotriazine

ROS: Reactive oxygen species

THF: Tetrahydrofuran

ACKNOWLEDGEMENTS

We are grateful for financial support from the Research Council of Alzahra University and support of National Elites Foundation of Iran, Tehran.

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