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EXCEEDINGLY FACILE ONE-POT PROTOCOLS TO THE SYNTHESIS OF PYRIMIDO ANNULATED ANALOGUES OF CARBAZOLO CONDENSED AZEPINONES AND THEIR EVALUATION FOR ANALGESIC ACTIVITY

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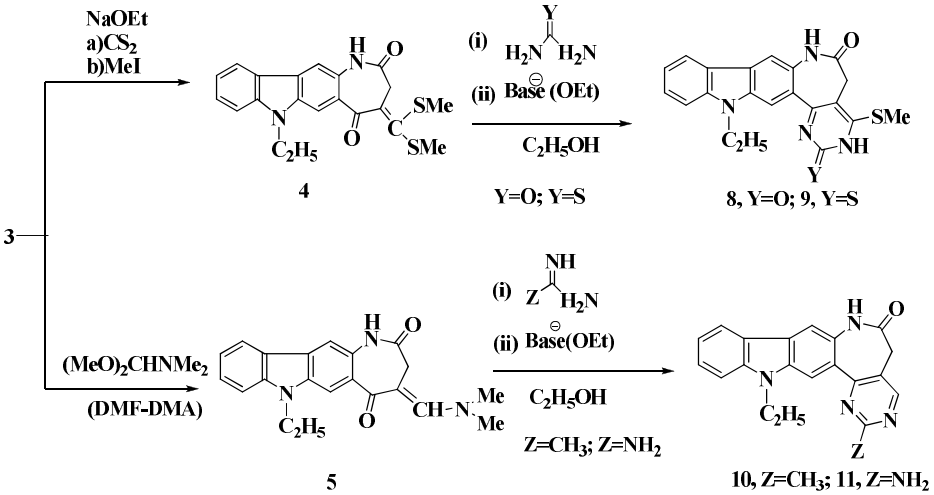
ABSTRACT. Extremely simple protocols based on the reactivity of corresponding oxoketenedithioacetal (**4**), 2-(dimethylaminomethylene) ketone (**5**), β-oxoenolether (**6**) and α,β-unsaturated ketone (**7**) derivatives of 7-ethyl-3, 4-dihydroazepino[3,2-b]carbazol-2,5(1H,7H)-dione (**3**) have been developed to provide an easy access to their pyrimido annulated analogues (**8-15**) of medicinal interest. The key compound **3** from which, the synthesis proceeded has been realized in two steps from the commercial 3-amino-9-ethyl carbazole (**1**) on its reaction in the first step with ethyl succinyl chloride followed by cyclocondensation of the resulting ester **2** with PPA. The selected synthesized compounds were screened for *in-vivo* analgesic activity using acetic acid induced writhing model in mice. Among them, compound **13**was found to be most active and found comparable to standard aspirin.

KEY WORDS: 3-Amino-9-ethyl carbazole, Oxoketnedithioacetal, Dimethyl formamide dimethyl acetal, 2-(Dimethylaminomethylene)ketone, Oxoenolether, Chalcone

INTRODUCTION

Carbazoles, benzazepines and pyrimidines exhibits a wide array of biological responses in combating a variety of body ailments by virtue of their ability to provide ligands to a number of functionally and structurally discrete receptors. Current demonstrations revealed that some of their derivatives can serve as potential agents in the treatment of cancer and AIDS has stimulated further interest in these molecules from yet another perspective.



Scheme 1

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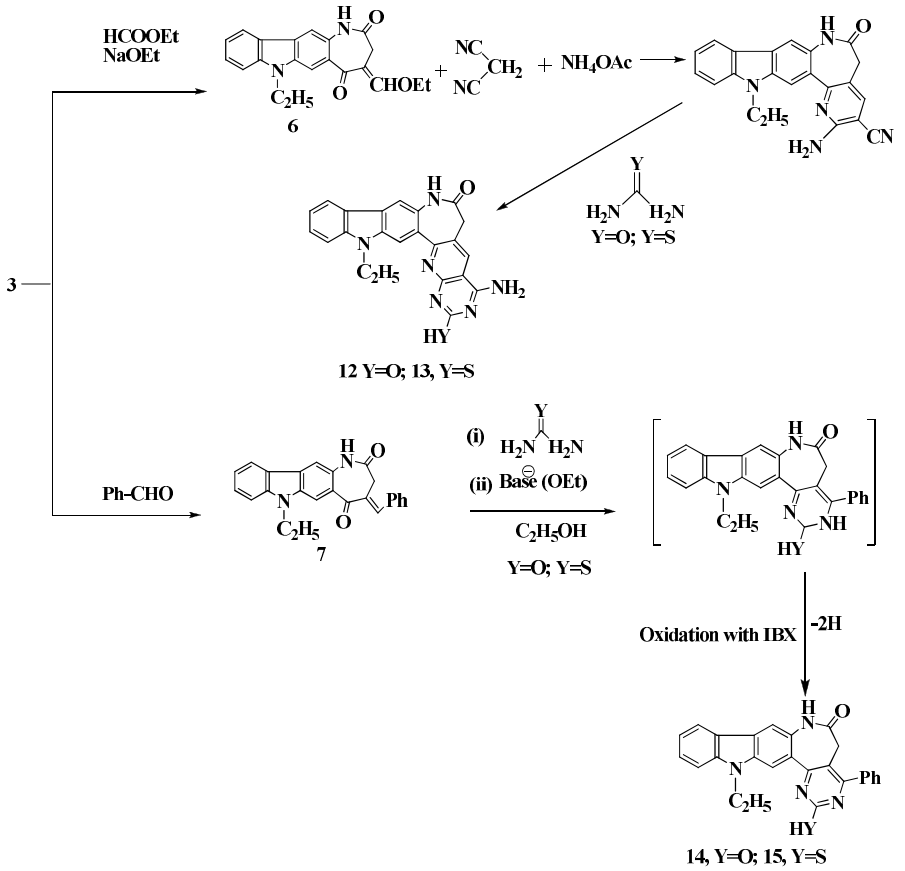
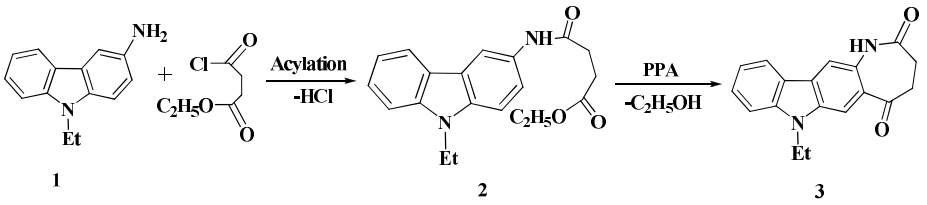


Scheme 2



Scheme 3

Literature is replete with examples showing that heterocycles which incorporate carbazoles [1], azepinones [2] and pyrimidines [3] in their molecular framework display wide range of bio-efficacies such as anti-cancer [4], anti-leukemic [5], antiproliferative [6], anti-microbial [7], anti-viral [8], anti-malarial [9], antioxidant [10], etc. Ever since, pyrimidine derivatives have been recognized to belong to the class of 'privileged medicinal scaffolds' [11] its potential have been widely exploited in the discovery of such pyrimidine based drugs as 'Etravirine' [12] which has emerged as a highly potent anti-HIV agent, to have found the FDA approval for the treatment of AIDS. The advent of its inherent potential such as this, has led to the interest on the

various facets of the chemistry of pyrimidine derivatives has expand exponentially, thereafter. In view of exploring, their biological potential further, we thought that it could be interesting to develop libraries ofsuch materials which contained a carbazole nucleus on one side of the azepinone framework and the bioactive pharmacophore such as the pyrimidine on its other side, on this premise, that their presence in tandem in the same molecular framework could contribute significantly to produce novel series of compounds with enhanced biological activities. In putting this concept into the action we require to develop expedient protocols to obtain the pyrimidine annulated analogues of azepino condensed carbazole derivatives **8-15** using conventional as well as microwave assisted methods. We explored the feasibility of their preparation by exploiting the synthetic potentials of oxoketenedithioacetal, 2-dimethyl-aminomethylene ketone, β-oxoenolether and α,β-unsaturated ketone derivatives **4-7** in their synthesis through their reaction with the bidentate nucleophiles, indicated in Scheme 1 and Scheme 2. These highly reactive systems having an exceptionally high propensity in reactions towards these nucleophiles were appended on to the adjacent position, of the carbonyl function in **3**, in accordance to the procedures reported for their incorporations in the literature on related substrates. The intermediate **3** was in turn realized through the procedure outlined in Scheme 3.

EXPERIMENTAL

General

Melting points were determined in an open capillary and are uncorrected. The IR spectra were recorded on Schimadzu FTIR-8400S. ^{1}H-NMR spectra were recorded in $CDCl_3$ on Bruker DRX-300 MHz spectrometer using TMS as internal reference with their values expressed in δ ppm. Purity of all the synthesized compounds were checked by TLC on silica gel G plates in the solvent system (9:1, benzene: methanol).

*Preparation of ethyl 4-(9-ethyl-9H-carbazol-3-ylamino)-4-oxobutanoate (**2**)*

To 3-amino-9-ethyl carbazole (**1**) (2.10 g, 0.01 mol) in dry pyridine (5.0 mL) was added ethyl succinyl chloride (1.64 g, 0.01 mol). The mixture was refluxed for 10-15 min. Cooled reaction mixture was poured slowly with stirring to 150-200 mL ice cold water. The solid which settled was filtered and washed with cold water and recrystallized from hot water containing a few drops of methanol to give **2** "in yield 2.82 g" (83%), m.p. 89-90 ^{o}C.

*Preparation of 7-ethyl-3,4-dihydroazepino [3,2-b] carbazol-2,5(1H, 7H)-dione (**3**)*

To ethyl 4-(9-ethyl-9H-carbazol-3-ylamino)-4-oxobutanoate (**2**) (2.58 g, 0.006 mol) PPA 20.0 g was added and the mixture was heated at 150-160 ^{o}C for 4 h. (the progress of the reaction was monitored by TLC). The reaction mixture was cooled to room temperature and a concentrated solution of Na_2CO_3 in water was added to make it neutral. The product was extracted with ethyl acetate (3x10 mL). The combined extracts were dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography on silica gel with $CHCl_3$ as an eluant to give **3**.

*Preparation of 4-(bis(methylthio)methylene)-7-ethyl-3,4-dihydroazepino[3,2-b]carbazol-2,5(1H,7H)-dione (**4**)*

To a mixture of 7-ethyl-3,4-dihydroazepino[3,2-b]carbazol-2,5(1H,7H)-dione (**3**) (1.75 g, 0.006 mol) and CS_2 (1 mL, 0.006 mol) was added a well stirred and cooled suspension of t-BuOK (1.34 g, 0.012 mol) in dry benzene (4.0 mL) and DMF (3.0 mL). The reaction mixture was allowed to stand at room temperature for 4 h. Methyl iodide (2.0 mL, 0.012 mol) was gradually

added with stirring and with external cooling (exothermic reaction). The reaction mixture was allowed to stand for further 4 h. at room temperature with occasional shaking. It was then refluxed on a water bath for 3 h. The mixture was poured on to the crushed ice and the benzene layer was collected. The aqueous portion was extracted with benzene and the combined extracts were washed, with water dried over anhydrous sodium sulfate. The solvent was removed in vacuum to give **4**.

*Preparation of (E)-4-((dimethylamino) methylene-7-ethyl-3,4-dihydroazepino[3,2-b]carbazol-2,5(1H,7H)-dione (**5**)*

7-Ethyl-3,4-dihydroazepino[3,2-b]carbazol-2,5(1H,7H)-dione **3** (2.92 g, 0.01 mol) was dissolved in N,N-dimethylformamide dimethylacetal (15 mL) and the solution was heated under reflux for 4 h. and concentrated. The residue is triturated with hexane, filtered and washed with hexane to give 5 as a powder (2.04 g).

*Preparation of 4-(ethoxymethylene)-7-ethyl-3,4-dihydroazepino[3,2-b]carbazole-2,5(1H,7H)-dione (**6**)*

To a suspension of sodium methoxide (0.5 g) in dry benzene (25.0 mL) at 0 ^{o}C, a solution of ethyl formate (0.74 g, 10 mmol) in dry benzene (10.0 mL) was added. To this mixture, 7-ethyl-3,4dihydroazepino[3,2-b]carbazol-2,5(1H,7H)-dione (**3**) (1.46 g, 0.005 mol) in benzene (10.0 mL) was then added. The mixture was stirred for 4 h. at room temperature and allowed to stand overnight. It is then diluted with cold water, acidified with dil. HCl and extracted with ether. The solvent was evaporated and the resulting compound was recrystallized from ethanol to give the pure product **6**, (1.08 g).

*Preparation of (Z)-4-benzylidene-7-ethyl-3,4-dihydroazepino[3,2-b]carbazole-2,5(1H,7H)-dione (**7**)*

A mixture of 7-ethyl-3,4-dihydroazepino[3,2-b] carbazol-2,5(1H,7H)-dione (**3**) (2.92 g, 0.01 mol), benzaldehyde (1.06 g, 0.01 mol) and fused sodium acetate (1.8 g, 0.015 mol) in glacial acetic acid (10 mL) was refluxed for 5 h. The reaction mixture was cooled in ice water. The crude solid was filtered, washed with water and recrystallized from aqueous ethanol to give **7**, (2.30 g) m.p. 72-73 ^{o}C.

*Preparation of 2-hydroxy-4-(methylthio)-5,7-dihydro-2H-N-ethylcarbazolo[b]pyrimido[4,5-d]azepin-6(3H)-one (**8**)*

To a mixture of urea (0.12 g, 0.002 mol), sodium ethoxide (0.14 g, 0.002 mol) and ethanol (25.0 mL) was added 4-(bis(methylthio)methylene)-7-ethyl-3,4-dihydroazepino[3,2-b]carbazol-2,5(1H,7H)-dione (**4**) (0.79 g, 0.002 mol). The reaction mixture was refluxed for 13-14 h. The solvent was removed by distillation and the residue was treated with glacial acetic acid (4-5 mL) (just enough to dissolve sodium salt of pyrimidine) and refluxed for 15 min. It was poured on crushed ice and the precipitated of **8** was purified by crystallization with chloroform (450 mg), m.p. 190-191 ^{o}C.

*Solution phase microwave assisted method for the preparation of (**8**)*

Urea (0.12 g, 0.002 mol), sodium ethoxide (0.14 g, 0.002 mol) and 4-(bis (methylthio)methylene)-7-ethyl-3,4-dihydroazepino[3,2-b]carbazole-2,5(1H,7H)-dione (**4**) (0.79 g, 0.002 mol) was taken in ethanol (5.0 mL). It was placed in a 100 mL borosil flask fitted with a funnel as a loose top. The reaction mixture was subjected to microwave irradiation at 180 W microwave power for 2 min followed by 360 W for 5 min and then at 720 W for 2 min (a short

interval of 1 min to avoid the excessive evaporation of solvents). Solid was treated with glacial acetic acid (4-5 mL) (just enough to dissolve sodium salt of pyrimidine) and refluxed to microwave irradiation at 180 W microwave power for 1 min and 360 W for 2 min. The overheating of the solution was avoided. The reaction mixture was poured on crushed ice and precipitated **8** was purified by crystallization with chloroform (350 mg).

*Preparation of 2-mercapto-4-(methylthio)-5,7-dihydro-2H-N-ethyl carbazolo[b]pyrimido [4,5-d] azepin-6(3H)-one (**9**)*

To a mixture of thiourea (0.152 g, 0.002 mol), sodium ethoxide (0.14 g, 0.002 mol) in ethanol (25.0 mL) was added 4-(bis(methylthio)methylene)-7-ethyl-3,4-dihydroazepino[3,2-b]carbazole-2,5(1H,7H)-dione (**4**) (0.79 g, 0.002 mol). The reaction mixture was refluxed for 13-14 h. The solvent was removed by distillation. The residue was treated with glacial acetic acid (4-5 mL) (just enough to dissolve sodium salt of pyrimidine) and refluxed for 15 min. The reaction mixture was poured on crushed ice and precipitated **9** was purified by crystallization with chloroform (460 mg).

*Solution phase microwave assisted method for the preparation of (**9**)*

Equimolar quantities of thiourea (0.152 g, 0.002 mol), sodium ethoxide (0.14 g, 0.002 mol) and 4-(bis(methylthio)methylene)-7-ethyl-3,4-dihydroazepino[3,2-b]carbazol-2,5(1H,7H)-dione (**4**) (0.79 g, 0.002 mol) were taken in ethanol (5.0 mL) and was placed in a 100 mL borosil flask fitted with a funnel as a loose top. The reaction mixture was subjected to microwave irradiation at 180 W microwave power for 2 min, 360 W for 5 min and then at 720 W for 2 min (with short interval of 1 min. to avoid the excessive evaporation of solvents). The residue was treated with glacial acetic acid (4-5 mL) (just enough to dissolve sodium salt of pyrimidine) and refluxed in a microwave at 180 W microwave power for 1 min. and 360 W for 2 min. The overheating of the solution was avoided. The reaction mixture was poured on crushed ice and precipitated **9** was purified by crystallization with chloroform (400 mg), m.p. 210-212 ^{o}C.

*Preparation of 2-methyl-5H-N-ethyl carbazolo[b]pyrimido[4,5-d]azepin-6(7H)-one (**10**)*

To a solution of (E)-4-((dimethylamino)methylene-7-ethyl-3,4-dihydroazepino[3,2-b]carbazole-2,5(1H,7H)-dione (**5**) (0.115 g, 333 mmol) in ethanol (10.0 mL) was added acetamidine hydrochloride (0.158 mg, 1.67 mmol) and Et_3N (2.35 g, 1.69 mmol). The solution was heated under reflux for 42 h and concentrated. The residue was extracted with AcOEt and washed with water. The organic layer was dried over anhydrous $MgSO_4$. The residue was purified by column chromatography on silica gel and eluted with hexane: AcOEt (1:2) to give brown crystals of **10** (68 mg).

*Solution phase microwave assisted method for the preparation of (**10**)*

(E)-4-((Dimethylamino)methylene-7-ethyl-3,4-dihydroazepino[3,2-b]carbazole-2,5(1H,7H)-dione (**5**) (0.115 g, 333 mmol) in ethanol (10.0 mL) was added acetamidine hydrochloride (0.158 g, 1.67 mmol) and Et_3N (2.35 g, 1.69 mmol). The mixture was placed in a 100 mL borosil flask fitted with a funnel as a loose top. The reaction mixture was subjected to microwave irradiation at 360 W for 6 min and then at 720 W for 3 min (with short interval of 1 min to avoid the excessive evaporation of solvents). The residue was extracted with AcOEt and washed with water. The organic layer was dried over anhydrous $MgSO_4$. The residue was purified by column chromatography on silica gel and eluted with hexane: AcOEt (1:2) to give brown crystals of **10** (72 mg).

*Preparation of 2-amino-5H-N-ethyl carbazolo[b]pyrimido[4,5-d]azepin-6(7H)-one (**11**)*

To a solution of (E)-4-((dimethylamino)methylene-7-ethyl-3,4-dihydroazepino[3,2-b]carbazol-2,5(1H,7H)-dione (**5**) (0.115 g, 333 mmol) in ethanol (25.0 mL) were added guanidine carbonate (1.5 g., 66.5 mmol) and sodium acetate (2.6 g, 0.04 mole). The mixture was heated under reflux for 56 h. The reaction mixture was filtered insoluble material was extracted with chloroform and washed with water. The organic layer was dried over anhydrous $MgSO_4$ and evaporated to give **11**, (70 mg), m.p. 165-167 ^oC.

Solution phase microwave assisted method for the preparation of (11)

Solution of (E)-4-((dimethylamino)methylene-7-ethyl-3,4-dihydroazepino[3,2-b]carbazol-2,5(1H,7H)-dione (**5**) (0.115 g, 0.333 mmol) in ethanol was added guanidine carbonate (1.5 g, 66.5 mmol) and sodium acetate (2.6 g, 0.04 mole). The mixture was placed in a 100 mL borosil flask fitted with a funnel as a loose top. The reaction mixture is subjected to microwave irradiation at 360 W for 6 min and then at 720 W for 3 min (with short interval of 1 min to avoid the excessive evaporation of solvents). The reaction mixture was filtered; insoluble material was extracted with chloroform and washed with water. The organic layer was dried over anhydrous $MgSO_4$ and evaporated to give **11** (75 mg).

*Preparation of 4-amino-7H-N-ethylcarbazolo[b]pyridopyrimidin-2-ol [2, 3-d] azepine-9(10H)-one (**12**)*

The solution of 7-ethyl-4-(hydroxymethylene)-3,4-dihyroazepino[3,2-b]carbazol-2,5(1H,7H)-dione (**6**) (1.74 g, 0.005mol), malononitrile (0.33 g, 0.005mol) and ammonium acetate (0.04 mol) in ethanol (10ml) was refluxed on a water bath for 20-22 h. The reaction mixture was cooled and poured on the crushed ice with constant stirring. A solid mass obtained was washed with water and ethanol and then the mixture of this solid mass (0.01 mol) and urea (0.02 mol) was heated on an oil bath at 180 ^oC and finally the mixture was heated at 220 ^oC for 2 h. On cooling the product solidified, which was recrystallized from DMF-EtOH mixture (1:2) to give **12** (1.20 g).

*Solution phase microwave assisted method for the preparation of (**12**)*

The solution of 7-ethyl-4-(hydroxymethylene)-3,4-dihyroazepino[3,2-b]carbazol-2,5(1H,7H)-dione **(6)** (1.74 g, 0.005 mol), malononitrile (0.33 g, 0.005 mol) and ammonium acetate (0.04 mol) in ethanol (10 mL) was placed in a 100 mL borosil flask fitted with a funnel as a loose top. The reaction mixture was subjected to microwave irradiation at 180 W microwave power for 3 min, 360 W for 4 min and then at 720 W for 3 min (with a short interval of 1 min to avoid the excessive evaporation of solvents). The reaction mixture was cooled and poured on the crushed ice with constant stirring. The solid mass thus obtained was washed with water and ethanol and then to this mixture of this solid mass (0.01 mol) and urea (0.02 mol) and heated in a microwave at 180 W microwave power for 1 min, 360 W for 2 min and then at 720 W for 1 min (with short interval of 1 min. to avoid the excessive evaporation of solvents). On cooling the product solidified, which was recrystallized from DMF-EtOH mixture (1:2) to give **12** (1.08 g).

*Preparation of 4-amino-7H-N-ethylcarbazolo[b]pyridopyrimidin-2-thiol[2,3-d]azepine-9 (10H)-one (**13**)*

The mixture of 7-ethyl-4-(hydroxymethylene)-3,4-dihyroazepino[3,2-b]carbazol-2,5(1H,7H)-dione (**6**) (1.74 g, 0.005 mol), malononitrile (0.33 g, 0.005 mol) and ammonium acetate (0.04 mol) in ethanol (10 mL) was refluxed on a water bath for 20-22 h. The reaction mixture was cooled and poured on the crushed ice with constant stirring. The solid mass obtained was

washed with water and ethanol and then to this mixture of this solid mass (0.01 mol) was added thiourea (0.02 mol) and heated on an oil bath at 180 ^{o}C and finally the mixture was heated at 220 ^{o}C for 2 h. On cooling the product solidified, which was recrystallized from DMF-EtOH mixture (1:2) to give **13** (1.14 g).

*Solution phase microwave assisted method for the preparation of (**13**)*

The mixture of 7-ethyl-4-(hydroxymethylene)-3,4-dihyroazepino[3,2-b]carbazole-2,5(1H,7H)-dione (**6**) (1.74 g, 0.005 mol), malononitrile (0.33 g, 0.005 mol) and ammonium acetate (0.04 mol) in ethanol (10 mL) was placed in a 100 mL borosil flask fitted with a funnel as a loose top. The reaction mixture was subjected to microwave irradiation at 180 W microwave power for 3 min., 360 W for 4 min and then at 720 W for 3 min (with short interval of 1 min to avoid the excessive evaporation of solvents). The reaction mixture was cooled and poured on the crushed ice with constant stirring. The solid mass thus obtained was washed with water and ethanol and then to this mixture was added thiourea (0.02 mol) and heated to microwave at 180 W microwave power for 1 min., 360 W for 2 min and then at 720 W for 1 min (with a short interval of 1 min to avoid the excessive evaporation of solvents). On cooling the product solidified, which was recrystallized from DMF-EtOH mixture (1:2) to give **13** (1.06 g).

*Preparation of 2-hydroxy-4-phenyl-5,7-dihydro-2H-N-ethylcarbazolo[b]pyrimido[4,5-d]azepin-6(3H)-one (**14**)*

The mixture of (Z)-4-benzylidene-7-ethyl-3,4-dihydroazepino[3,2-b]carbazol-2,5(1H,7H)-dione (**7**) (0.380 g, 0.001 mol), urea (0.11 g, 0.001 mol) and NaOH (0.1 g) in 25 mL of 80% dilute ethanol was refluxed for 1.5 h. The reaction mixture was concentrated, and cooled, the precipitate obtained was filtered off and recrystallized from DMF/water to give **14 (**260 mg).

*Preparation of 2-mercapto-4-phenyl-5,7-dihydro-2H-N-ethyl carbazolo[b]pyrimido[4,5-d]azepin-6(3H)-one (**15**)*

The mixture of (Z)-4-benzylidene-7-ethyl-3,4-dihydroazepino[3,2-b]carbazole-2,5(1H,7H)-dione (**7**) (0.380 g, 0.001 mol), thiourea (0.01 g, 0.001 mol) and NaOH (0.1 g) in 25 mL of 80% dilute ethanol was refluxed for 1.5 h. The reaction mixture was concentrated, cooled and the precipitate obtained was filtered off and recrystallized from DMF/water to give **15** (250 mg).

Evaluation of analgesic activity

Acute toxicity studies were carried out in mice in accordance to OECD-420 guidelines [13]. Albino mice (20-25 g) either sex were divided into six groups of containing six animals each. Animals were starved for 24 h with water ad libitum prior to test. On the day of the experiment, animals were administered with different test compounds to different groups in graded doses of 10-1000 mg/kg body weight orally. The animals were then observed continuously for 3 h for general behavioral, neurological, autonomic profiles and then every 30 min for next 3 h and also for incidences of mortality if any.

Peripheral analgesic activity - acetic acid induced writhing test in mice

Mice were divided into six groups containing six each mice. The control group received normal saline (2 mL/kg, I.P.). The test groups were treated with compounds (10 mg/kg I.P.) while the second group received aspirin at the dose of 10 mg/kg I.P. After 30 min of compound administration, the mice were challenged with 0.6% acetic acid intraperitoneally (10 mL kg^{-1}) [14]. Five minutes after acetic acid injection, mice were placed in individual cage and the

number of abdominal contractions was counted for each mouse for a period of 10 min after 5 min latency, and the percentage inhibition of writhing was calculated.

The percentage inhibition was calculated by using the formula:

Percentage inhibition = $(N_C - N_T/N_C) \times 100$

Where N_T is average number of writhing in treated group and N_C is average number of writhing in control group.

RESULTS AND DISCUSSION

As a part of an ongoing endeavour to develop novel heterocyclic scaffolds of anticipated biological activity [15-17] from easily accessible starting materials, we report herein in this communication, the preliminary results of our study on the synthesis of pyrimidine annulated analogues of carbazolo fused azepinones (**8-15**) from 7-ethyl-3,4-dihydroazepino[3,2-b] carbazol-2,5(1H,7H)-dione (**3**) [18]. A perusal of literature on the preparation of oxoketenedithioacetals, 2-(dimethylaminomethylene) ketones, oxoenolethers and α,β-unsaturated ketones (chalcones), demonstrated that these were readily available from the base catalyzed condensation of carbonyl species containing an active methylene group with (i) CS_2+ MeI (ii) DMF-DMA (iii) HCOOEt and (IV) C_6H_5CHO respectively. Application of this strategy on **3** afforded the intermediates **4-7** in moderate to good yield. The versatility of these novel precursors in heteroannulations of **3** was examined by allowing **4-7** derivatives to react with urea, thiourea, acetamidine and guanidine, which resulted the desired pyrimidine annulated analogues (**8-15**) in acceptable yields. Compound **3** was in turn realized from the commercial 3-amino-9-ethyl carbazole (**1**) on its reaction with ethyl succinyl chloride under the conditions of Friedel-Crafts acylation, followed by cyclocondensation of the resulting intermediate with PPA. The physical analytical and spectral data of the compounds are given in Table 1 and 2. All the synthesized compounds gave satisfactory results for elemental analysis. IR and ^{1}H-NMR spectral data which were found to be consistent to the structures assigned to these molecules.

*Physical, analtical and spectral data of compounds **3-15***

3. Yield, 59%, m.p. 158-160 ^{o}C. IR (KBr) cm^{-1} 3340 [NH str], 2946 [C-H str ArH], 1704 [C=O str], 1712 [C=O str], 1458 [C-H str CH_3], 1072 [C-N str]. ^{1}H-NMR ($CDCl_3$) δ 8.0 (1H, s, NH), 7.78 (1H, s, CH), 7.63 (1H, s, CH), 7.55 (1H, d, CH, J = 6.2), 7.40 (1H, d, CH, J = 6.4), 7.08 (1H, t, CH, J = 6.5, 6.1), 7.00 (1H, t, CH, J = 6.4, 6.1), 3.89 (2H, q, CH_2), 2.93 (2H, t, CH_2, J = 7.1, 7.0), 2.42 (2H, t, CH_2, J = 8.3, 8.2), 1.51 (3H, t, CH_3, J = 7.3, 7.5). ^{13}C-NMR ($CDCl_3$) δ 14.4, 27.5, 34, 38, 109, 110, 111, 112, 118, 119, 120, 122, 125, 128, 131,134, 171, 200. MS: [m/z] 292 (19%, M). Anal. calcd./found for $C_{18}H_{16}N_2O_2$: C, 73.95/73.93; H, 5.52/5.49; N, 9.58/9.62.

4. Yield, 47%, m.p. 74-75 ^{o}C. IR (KBr) cm^{-1} 1690 [C=O str], 1705 [C=O str], 1020 [C-N str], 680 [C-S str]. ^{1}H-NMR ($CDCl_3$)δ, 8.0 (1H, s, NH), 7.74 (1H, s, CH), 7.70 (1H, s, CH), 7.55 (1H, d, CH, J= 6.1), 7.40 (1H, d, CH, J = 6.5), 7.08 (1H, t, CH, J = 6.2, 6.3), 7.00 (1H, t, CH, J = 6.7, 6.5), 3.89 (2H, q, CH_2), 2.90 (2H, s, CH_2), 2.25 (3H, s, CH_3), 3.25 (3H, s, CH_3), 1.51 (3H, t, CH_3, J = 7.5, 7.6). ^{13}C-NMR ($CDCl_3$) δ 14.6, 18, 18.5, 21, 37, 103, 109.5, 110.4, 111, 112, 119.8, 120.5, 121, 123,125, 126, 135, 155, 165, 187, 193 MS: [m/z] 396 (25%, M). Anal. calcd./found for $C_{21}H_{20}N_2O_2S_2$: C, 63.61/63.63; H, 5.08/5.05; N, 7.06/7.13; S, 16.17/16.25.

5. Yield, 58%, m.p. 80-81 ^{o}C. IR (KBr) cm^{-1} 1680 [C=O str], 1710 [C=O str], 1050 [C-N str], 815 [*o*-monosub]. ^{1}H-NMR ($CDCl_3$) δ, 8.0 (1H, s, NH), 7.74 (1H, s, CH), 7.70 (1H, s, CH), 7.55 (1H, d, CH, J = 6.1), 7.40 (1H, d, CH, J = 6.2), 7.26 (1H, s, CH), 7.08 (1H, t, CH, J = 6.8, 6.7), 7.00 (1H, t, CH, J= 6.5, 6.3), 3.89 (2H, q, CH_2), 2.90 (2H, s, CH_2), 2.47 (6H, s, CH_3), 1.51 (3H,

t, CH_3, J = 7.5, 7.6). ^{13}C-NMR ($CDCl_3$) δ 14.6, 27, 38, 43.5, 44.6, 103, 109, 110, 111, 111.7, 112, 119, 121, 125, 125.7, 126, 135.2, 136, 149, 168, 187. MS: [m/z] 347 (22%, M). Anal. calcd./found for $C_{21}H_{21}N_3O_2$: C, 72.60/72.62; H, 6.09/6.03; N, 12.10/12.08.

6. Yield, 62%, m.p. 90-91 °C. IR (KBr) cm^{-1} 1685 [C=O str], 1705 [C=O str], 1480 [C=C str ArH], 1120 [C-N str], 810 [*o*-monosub]. ^{1}H-NMR ($CDCl_3$) δ, 8.17 (1H, d, CH, J = 8.2), 8.12 (1H, s, CH), 8.04 (1H, s, NH), 7.93 (1H, s, CH), 7.68 (1H, s, CH), 7.59 (1H, d, CH, J = 8.1), 7.42 (2H, t, CH, J = 7.8, 6.1), 4.49 (2H, q, CH_2), 4.53 (2H, q, CH_2), 2.90 (2H, s, CH_2), 1.29 (3H, t, CH_3, J = 8.8, 1.9), 1.21 (3H, t, CH_3, J = 7.1, 1.8). ^{13}C-NMR ($CDCl_3$) δ 14.4, 15.3, 32, 37.6, 70.5, 104, 106, 108, 110, 112, 117, 119, 121, 121.7, 125, 126, 127, 135, 162, 168, 187. MS: [m/z] 348 (22%, M). Anal. calcd./found for $C_{21}H_{20}N_2O_3$: C, 72.40/72.36; H, 5.79/5.76 ; N, 8.04/ 8.06.

7. Yield, 60%, m.p. 72-73 °C. IR (KBr) cm^{-1} 1665 [C=O], 1710 [C=O], 1180 [C-N str], 860 [*o*-monosub]. ^{1}H-NMR ($CDCl_3$) δ, 8.0 (1H, s, NH), 7.74 (1H, s, CH), 7.70 (1H, s, CH), 7.55 (1H, d, CH, J = 7.2), 7.40 (1H, d, CH, J = 8.2), 7.30 (2H, d, CH, J = 7.5), 7.21 (2H, t, CH, J = 7.1, 1.8), 7.14 (1H, t, CH, J = 7.5, 1.7), 7.08 (1H, t, CH, J = 7.5, 1.8), 7.00 (1H, t, CH, J = 7.1, 6.2), 3.89 (2H, q, CH_2), 2.90 (2H, s, CH_2), 1.51 (3H, t, CH_3, J = 7.6, 6.8). ^{13}C-NMR ($CDCl_3$) δ 14, 28, 37.8, 104, 108, 112, 119, 119.8, 121, 122, 125, 127, 128, 128.6, 129, 135, 136, 138, 139, 168, 187. MS: [m/z] 380 (18%, M^+). Anal. calcd./found for $C_{25}H_{20}N_2O_2$: C, 78.93/78.91; H, 5.30/5.28; N, 7.36/7.40.

8. Yield, 57%, m.p. 190-191 °C. IR (KBr) cm^{-1} 3600 [O-H], 1665 [C=O], 1680 [C=O in pyrimidinone ring]. ^{1}H-NMR ($CDCl_3$) δ, 8.17 (1H, s, CH), 8.17 (1H, d, CH, J = 8.8), 8.02 (1H, s, CH), 8.0 (1H, s, NH), 7.59 (1H, d, CH, J = 8.3), 7.42 (2H, t, CH, J = 8.8, 6.5), 6.00 (1H, s, CH), 4.53 (2H, q, CH_2), 2.90 (2H, s, CH_2), 2.43 (3H, s, CH_3), 2.0 (2H, s, NH and OH), 1.29 (3H, t, CH_3, J = 8.1, 6.5). ^{13}C-NMR ($CDCl_3$) δ 14, 18, 28, 37, 96, 108, 103, 110, 111, 114, 120, 121.7, 125, 126, 134, 137, 150, 153, 156, 164.6. MS: [m/z] 392 (11%, M). Anal. calcd./found for $C_{21}H_{20}N_4O_2S$: C, 64.27/64.22; H, 5.14/5.12; N, 14.28/14.24; S, 8.17/8.21.

9. Yield, 56%, m.p. 210-212 °C. IR (KBr) cm^{-1} 2212 [S-H], 1665 [C=O], 1680 [C=O in pyrimidinone ring]. ^{1}H-NMR ($CDCl_3$) δ, 8.17 (1H, s, CH), 8.17 (1H, d, CH), 8.02 (1H, s, CH), 8.0 (1H, s, NH), 7.59 (1H, d, CH, J = 8.3), 7.42 (2H, t, CH, J = 8.8, 6.5), 5.33 (1H, s, CH), 4.53 (2H, q, CH_2), 2.90 (2H, s, CH_2), 2.43 (3H, s, CH_3), 2.0 (1H, s, NH), 1.5 (1H, s, SH), 1.29 (3H, t, CH_3, J = 8.1, 6.5). ^{13}C-NMR ($CDCl_3$) δ 14.2, 18.1, 27.5, 40.3, 86.3, 108, 103.3, 110, 111.8, 114, 120, 121.7, 125, 126, 134.5, 137, 150, 153, 156, 164.6. MS: [m/z] 408 (17%, M). Anal. calcd./found for $C_{21}H_{20}N_4OS_2$: C, 61.74/61.71; H, 4.93/4.95; N, 13.75/13.71; S, 15.70/15.63.

10. Yield, 59%, m.p. 121-122 °C. IR (KBr) cm^{-1} 1665 [C=O], 1581 [C=N str], 1242 [C-N str]. ^{1}H-NMR ($CDCl_3$) δ, 8.14 (1H, s, CH), 8.0 (1H, s, NH), 7.61 (1H, s, CH), 7.55 (1H, d, CH, J = 8.7), 7.40 (1H, d, CH, J = 8.8), 7.37 (1H, s, CH), 7.08 (1H, t, CH, J = 8.0, 6.4), 7.00 (1H, t, CH, J = 8.5, 6.7), 3.89 (2H, q, CH_2), 3.49 (2H, s, CH_2), 2.35 (3H, s, CH_3), 1.51 (3H, t, CH_3, J = 8.7, 6.2). ^{13}C-NMR ($CDCl_3$) δ 14, 24, 37, 44, 103, 106, 108, 109, 110, 111, 118, 119, 121, 125, 126, 127, 132, 157, 158, 164, 168. MS: [m/z] 342 (12%, M^+). Anal. calcd./found for $C_{21}H_{18}N_4O$: C, 73.67/73.63; H, 5.30/5.32; N, 16.36/16.40.

11. Yield, 61%, m.p. 165-167 °C. IR (KBr) cm^{-1} 1665 [C=O], 1580 [C=N str], 1210 [C-N str]. ^{1}H-NMR ($CDCl_3$) δ, 8.0 (1H, s, NH), 7.98 (1H, s, CH), 7.61 (1H, s, CH), 7.55 (1H, d, CH), 7.40 (1H, d, CH, J = 8.7), 7.37 (1H, s, CH), 7.08 (1H, t, CH), 7.00 (1H, t, CH, J = 8.0, 6.4), 4.0 (2H, s, NH_2), 3.89 (2H, q, CH_2), 3.49 (2H, s, CH_2), 1.51 (3H, t, CH_3, J = 8.7, 6.2). ^{13}C-NMR ($CDCl_3$) δ 14.6, 24.8, 37, 40.3, 44, 103.5, 106, 108, 109, 110, 111, 118, 119, 121.7, 124, 126.2, 127, 132.5, 157.8, 158, 164, 168.9. MS: [m/z] 343 (14%, M). Anal. calcd./found for $C_{20}H_{17}N_5O$:

C, 69.96/ 69.94; H, 4.99/4.97; N, 20.40/20.42.

12. Yields, 58%, m.p. 175-177 ^{o}C. IR (KBr) cm^{-1} 1660 [C=O], 980 [C-O-N str.], 3510 [O-H]. ^{1}H-NMR ($CDCl_3$) δ, 8.17 (1H, d, CH, J = 8.9), 8.11 (1H, s, CH), 8.02 (1H, s, CH), 8.0 (1H, s, NH), 7.82 (1H, s, CH), 7.59 (1H, d, CH, J = 8.2), 7.42 (2H, t, CH, J = 7.7, 6.0), 6.90 (1H, s, NH_2), 4.53 (2H, q, CH_2), 4.4 (1H, s, CH), 4.0 (1H, s, NH), 3.49 (2H, s, CH_2), 2.0 (2H, s, OH), 1.29 (3H, t, CH_3, J = 8.7, 6.2). ^{13}C-NMR ($CDCl_3$) δ 14, 37, 40, 44, 99, 102, 103, 109, 110, 111.2, 112, 120.9, 121, 122.7, 125, 126.2, 136, 138, 160, 164, 168.2 MS: [m/z] 412 (19%, M). Anal. calcd./found for $C_{23}H_{20}N_6O_2$: C, 66.98/66.67; H, 4.89/4.87; N,20.38/20.27.

13. Yields, 53%, m.p. 182-184 ^{o}C. IR (KBr) cm^{-1} 1650 [C=O], 1573 [C=N str], 725 [C-S str]. ^{1}H-NMR ($CDCl_3$) δ 8.17 (1H, d, CH, J = 8.9), 8.11 (1H, s, CH), 8.02 (1H, s, CH), 8.0 (1H, s, NH), 7.82 (1H, s, CH), 7.59 (1H, d, CH, J = 8.2), 7.42 (2H, t, CH, J = 7.7, 6.0), 6.90 (1H, s, NH_2), 4.53 (2H, q, CH_2), 3.7 (1H, s, CH), 4.0 (1H, s, NH), 3.49 (2H, s, CH_2), 1.5 (1H, s, SH), 1.29 (3H, t, CH_3, J = 8.7, 6.2). ^{13}C-NMR ($CDCl_3$) δ 14, 37, 40, 44, 99, 102, 103, 109, 110, 111.2, 112, 120.9, 121, 122.7, 125, 126.2, 136, 138, 160, 164, 168.2. MS: [m/z] 428 (11%, M). Anal. calcd./found for $C_{23}H_{20}N_6OS$: C, 64.47/64.50; H, 4.70/4.72; N,19.61/19.64; S, 7.48/7.44.

14. Yield, 62%, m.p. 90-91 ^{o}C. IR (KBr) cm^{-1} 3550 [O-H], 1670 [C=O]. ^{1}H-NMR ($CDCl_3$) δ 8.17 (1H, d, CH, J =7.7), 8.11 (1H, s, CH), 8.02 (1H, s, CH), 8.0 (1H, s, NH), 7.71 (2H, d, CH, J = 7.9), 7.59 (1H, d, CH, J = 8.1), 7.42 (2H, t, CH, J = 7.2, 5.9), 7.40 (2H, t, CH, J = 7.1, 6.3), 7.33 (1H, t, CH, J = 7.2, 6.5), 6.00 (1H, s, CH), 4.53 (2H, q, CH_2), 2.90 (2H, s, CH_2), 2.0 (2H, s, NH and OH), 1.29 (3H, t, CH_3, J = 7.7, 6.2). ^{13}C-NMR ($CDCl_3$) δ 14, 40.3, 42.1, 97, 103.3, 104, 109.7, 110, 111, 119, 121, 125, 126, 127, 128, 129.2, 129.5, 132.5, 133.0, 138, 153, 158, 164, 168.2.MS: [m/z] 422 (12%, M). Anal. calcd./found for $C_{26}H_{22}N_4O_2$: C, 73.92/73.88; H, 5.25/5.22; N,13.26/13.30.

15. Yield, 57%, m.p. 117-119 ^{o}C. IR (KBr) cm^{-1} 2180 [S-H], 1680 [C=O], 810 [C-S str]. ^{1}H-NMR ($CDCl_3$) δ, 8.17 (1H, d, CH, J =7.7), 8.17 (1H, s, CH), 8.02 (1H, s, CH), 8.0 (1H, s, NH), 7.71 (2H, d, CH, J = 7.9), 7.59 (1H, d, CH, J = 8.1), 7.42 (2H, t, CH, J = 7.2, 5.9), 7.40 (2H, t, CH, J = 7.1, 6.3), 7.33 (1H, t, CH, J = 7.2, 6.5), 5.33 (1H, s, CH), 4.53 (2H, q, CH_2), 2.90 (2H, s, CH_2), 2.0 (1H, s, NH), 1.5 (1H, s, SH), 1.29 (3H, t, CH_3, J= 7.7, 6.2).^{13}C-NMR ($CDCl_3$) δ 14, 40.3, 42.1, 97, 103.3, 104, 109.7, 110, 111, 119, 121, 125, 126, 127, 128, 129.2, 129.5, 132.5, 133.0, 138, 153, 158, 164, 168.2.MS: [m/z] 438 (20%, M). Anal. calcd./found for $C_{26}H_{22}N_4OS$: C, 71.21/71.25; H, 5.06/5.11; N,12.78/12.74.

Comparative analysis between conventional and microwave synthesis revealed us that microwave process allows access to a library of pyrimidine annulated analogues of carbazolo fused azepinones in very short reaction time without affecting the yield and purity of the target compounds (Table 1).

Table 1. Showing the difference between conventionally and microwave assisted reactions.

Compound	Time conventionally (h)	Yield (%)	Time microwave assisted (min)	Yield (%)
8	13-14	57	13	44
9	13-14	56	13	49
10	42	59	10	63
11	56	61	10	65
12	22-24	58	16	52
13	23-24	53	16	49

Preliminary acute toxicity studies revealed that test compounds were safe and did not cause any mortality up to a maximum dose of 1000 mg/kg body weight. The animals were physically

active and were consuming food and water in a regular way. But, few behavioral changes were observed in mice at higher doses in the response to test compounds like altered alertness, touch response, and locomotion. Therefore, 1/100th of the maximum tolerated dose, i.e. 10 mg/kg body weight (b.w.) was chosen for the studies.

The abdominal constriction response induced by glacial acetic acid is a sensitive procedure to establish peripherally acting analgesics. This response is thought to involve local peritoneal receptors. Acetic acid causes inflammatory pain by inducing capillary permeability and liberating endogenous substances that excite pain nerve ending. Acetic acid is also known to increase PGE_1 and PGE_2 peripherally [19]. In acetic acid induced writhing model, all the compounds (10 mg/kg) exhibited impressive analgesic activity *via* reduction of writhes in mice. It is noteworthy, that compound **13** caused maximum inhibition (80.1%, $p < 0.05$) and was comparable to standard (Table 2).

Table 2. Effect of synthesized compounds on acetic acid induced writhing in mice (n = 6).

Treatment (mg/kg)	Mean number of writhes	Percentage inhibition (%)
Control	63.0 ± 12*	00**
Aspirin (10 mg/kg)	8.0 ± 6.1*	87.3**
Compound **12** (10 mg/kg)	18.8 ± 1.4*	69.8**
Compound **13** (10 mg/kg)	12.3 ± 2.6*	80.1**
Compound **14** (10 mg/kg)	46.8 ± 3.2*	25.5**
Compound **15** (10 mg/kg)	24.5 ± 2.9*	57.7**

Each value represents mean ± SEM. *$p < 0.05$ compared with control (student's t-test) n = 6). **$p < 0.001$.

NSAIDs can inhibit COX in peripheral tissues and therefore interfere with the mechanism of transduction of primary afferent nociceptors [20]. The mechanism of analgesic activity of compounds could be probably due to the blockade of the effect or the release of endogenous substances that excite pain nerve endings similar to that of aspirin and NSAIDs. Thus, the reduction in the number of writhing by the test compounds indicates that compounds might exert analgesic activity by inhibition of liberating endogenous substances like prostaglandin and other cytokines that excite pain nerve ending and induces pain or via inhibition of arachidonic acid pathway or their key enzymes like cyclooxygenases. Thus results obtained in this study indicate that test compounds possess analgesic properties probably via peripheral inhibitory mechanisms.

CONCLUSION

In summary, several elegant protocols have been developed to provide an easy access to the novel pyrimido annulated analogues of carbazolo condensed azepinone derivatives (**8-15**) from the corresponding oxoketenedithioacetal, 2-(dimethylaminomethylene) ketone, β-oxo-enolether and α, β-unsaturated ketones (**4**, **5**, **6** and **7**), respectively, in high yield and purity. Among all test compounds, compound **13** displayed most profound analgesic effect and found comparable to standard. Further studies in depth toxicity studies and PK-PD studies could to be helpful in designing a more potent analgesic for therapeutic use in the field of medical science.

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