World Journal of Chemistry 5 (1): 07-17, 2010
ISSN 1817-3128

Synthesis, Anticancer and Anti-Inflammatory Activities of 3, 4-Dihydro-7-nitrobenzo[*b*]oxepin-5(2*H*)-one and its Related Derivatives

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Abstract: A series of pyridine, pyrazole and pyrimidine derivatives were newly synthesized using 3,4-dihydro-7-nitrobenzo[*b*]oxepin-5(2*H*)-one 2 as a starting material. The anticancer activities of the synthesized products were evaluated following the known *in vitro* disease oriented DTP-one dose human cancer cell lines screening program, which is based upon the use of one dose at 10^{-4} concentration against multiple panels of 60 human tumor cell lines which are derived from nine types (lung, colon, breast, ovarian, leukemia, renal, melanoma, prostate and CNS) by the National Cancer Institute, USA. Some of the compounds showed moderate inhibitory effects on the growth of the tested cancer cell lines. The detailed synthesis, spectroscopic data and antitumor properties for the synthesized products are reported.

Key words: Nitrobenzo[*b*]oxepine · Anticancer · Anti-inflammatory

INTRODUCTION

In our previous work, we have found that some of our newly substituted heterocyclic compounds exhibited antimicrobial, anti-inflammatory and anticancer activities [1-6]. Also, it was reported that the benzoxepine moiety plays a core structure both in naturally occurring products and in certain synthetic biological molecules. They are known to exhibit pharmacological activities such as vasodilators, anti-implantation, anti-inflammatory, antipyretic, analgesic agents [7-9]. Pyrazoles present an interesting group of compounds many of which possess widespread pharmacological properties such as analgesic, antipyretic and antirheumatic activities [10, 11]. In addition, the pharmacological and antitumor activities of many compounds containing nitrogen heterocyclic ring such as pyridine and pyrimidine have been reviewed [12-16]. In view of these reports and in continuation of our previous works in heterocyclic chemistry, we have herein synthesized some new derivatives containing heterocyclic ring fused with substituted benzoxepine moiety for the evaluation of their anticancer activity.

MATERIALS AND METHODS

Melting points were determined in open glass capillaries using an Electrothermal IA 9000 SERIES digital melting point apparatus (Electrothermal UK) and are uncorrected. Microanalyses were performed for all final compounds on an Elementar-Vario EL (Elementar-Vario EL, Germany) (Micro-analytical Unit, Central Services Laboratory, National Research Centre, Cairo; Egypt). Analyses of C, H, N and S were found to be within acceptable limits of theoretical values. The ^{1}H NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer (Varian, USA). ^{1}H NMR spectra were run at 300 MHz in $CDCl_3$ and DMSO-d_6 as solvent. Chemical shifts δ are quoted in ppm and were related to that of the solvents. Mass spectra were recorded on a Shimadzu GCMS-QP 1000EX (EI, 70 eV) (Shimadzu, Japan) and Hewlett-Packard (EI, 70 eV) (Hewlett-Packard, USA). IR spectra were obtained with a Bruker-Vector 22 (Bruker, Rheinstetten, Germany). All the reactions were monitored using thin layer chromatography (TLC) using silica gel aluminum sheets $60F_{254}$ (Merck).

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3,4-Dihydrobenzo[*b*]oxepin-5(2*H*)-one (1): Prepared from Phenol and γ-buterolactone to get 4-phenoxybutanoic acid, followed by cyclization using PPA to get 1 in (85%), m.p. 64-65°C (lit. [17]: yield 70%, m.p. 63°C) according to Traynelis and Love as yellow syrup and used without further purification.

3, 4-Dihydro-7-nitrobenzo[*b*]oxepin-5(2*H*)-one (2):
Method (A): Compound 1 (6.48 g, 40 mM), was added to a stirred cold solution of fuming nitric acid over a period of 30 min, the stirred reaction mixture was kept at low temperature (-10°C) for another 30 min, poured onto ice-cooled water and the obtained solid was filtered off, washed thoroughly with water, dried and crystallized from ethanol To give 2. Yield 5.1 g (62%), m.p. 119-121°C (EtOH); IR (KBr, cm^{-1}): 1716 (C=O); ^{1}H NMR (DMSO-d_6) δ: 2.20-2.22 (m, 2H, CH_2-oxepin), 2.87 (t, 2H, CH_2-oxepin), 4.36-4.38 (t, 2H, CH_2-oxepin), 7.29 (d, 1H, Ar-H), 8.28 (d, 1H, Ar-H), 8.37 (s, 1H, Ar-H); MS m/z (%): 207 [M^+] (32), corresponding to the molecular formula $C_{10}H_9NO_4$ and base peak at 63 (100).

Method (B): Compound 1 (3.2 g, 20 mM), was added to a stirred cold (-10°C) solution of conc. H_2SO_4 (30 ml). A mixture of (15 ml conc. H_2SO_4 with 1.5 ml nitric acid 65%) was added dropwise over a period of 30 min at the same temperature. Then stirring was maintained for additional 1h at room temperature. Then the reaction mixture was poured onto ice-cooled water and the formed solid was filtered off, washed, dried to give compound 2 (2.5 g, 60% yield) as identified by its TLC behavior, mp and mixed mp with an authentic sample from Method A.

Synthesis of Benzylidene Derivatives (3a-g)
General Method: To a mixture of the ketone 2 (1.04 g, 5 mM) and the appropriate aromatic aldehydes (5 mM) [4-fluorobenzaldehyde (0.62 g), 4-chlorobenzaldehyde (0.71 g), 4-bromobenzaldehyde (0.93 g), 2-nitrobenzaldehyde (0.76 g), 4-tolualdehyde (0.61 g), 4-isopropylaldehyde (0.74 g) and 3,4,5-trimethoxybenzaldehyde (0.98 g)] in ethanol (50 ml), a mixture of sulphuric acid and glacial acetic acid (1:3) was added and the reaction mixture was stirred at room temperature for 1h. The formed solid was filtered off, washed thoroughly with water, aqueous ethanol, dried and crystallized from the appropriate solvent to give the corresponding benzylidene derivative 3.

4-(4-Fluorobenzylidene)-3,4-dihydro-7-nitrobenzo[*b*]-oxepin-5(2*H*)-one (3a): Yield 1.14 g (73%), m.p. 144-146°C (MeOH); IR (KBr, cm^{-1}): 1664 (C=O); ^{1}H NMR (DMSO-d_6) δ: 3.17-3.18 (t, 2H, CH_2-oxepin), 4.51-4.53 (t, 2H, CH_2-oxepin), 7.38-7.43 (m, 5H, Ar-H), 7.51 (s, 1H, CH), 8.30 (d, 1H, Ar-H), 8.53 (s, 1H, Ar-H); MS m/z (%): 313 [M^+] (18), corresponding to the molecular formula $C_{17}H_{12}FNO_4$ and base peak at 314 (100).

4-(4-Chlorobenzylidene)-3,4-dihydro-7-nitrobenzo-[*b*]oxepin-5(2*H*)-one (3b): Yield 1.14 g (73%), m.p. 153-155°C (EtOH); IR (KBr, cm^{-1}): 1661 (C=O); ^{1}H NMR (DMSO-d_6) δ: 3.16-3.18 (t, 2H, CH_2-oxepin), 4.50-4.51 (t, 2H, CH_2-oxepin), 7.42-7.47 (m, 5H, Ar-H), 7.51 (s, 1H, CH), 8.31 (d, 1H, Ar-H), 8.55 (s, 1H, Ar-H); MS m/z (%): 329 [M^+] (61), corresponding to the molecular formula $C_{17}H_{12}ClNO_4$ and base peak at 330 (100).

4-(4-Bromobenzylidene)-3,4-dihydro-7-nitrobenzo-[*b*]oxepin-5(2*H*)-one (3c): Yield 0.84 g (45%), m.p. 149-151°C (MeOH); IR (KBr, cm^{-1}): 1660 (C=O); ^{1}H NMR (DMSO-d_6) δ: 3.18-3.19 (t, 2H, CH_2-oxepin), 4.53-4.54 (t, 2H, CH_2-oxepin), 7.39-7.45 (m, 5H, Ar-H), 7.50 (s, 1H, CH), 8.29 (d, 1H, Ar-H), 8.53 (s, 1H, Ar-H); MS m/z (%): 375 [M^++1] (3), corresponding to the molecular formula $C_{17}H_{12}BrNO_4$ and base peak at 373 (100).

4-(2-Nitrobenzylidene)-3,4-dihydro-7-nitrobenzo-[*b*]oxepin-5(2*H*)-one (3d): Yield 0.95 g (55%), m.p. 169-171°C (dioxane/H_2O); IR (KBr, cm^{-1}): 1663 (C=O); ^{1}H NMR (DMSO-d_6) δ: 3.16-3.18 (t, 2H, CH_2-oxepin), 4.50-4.51 (t, 2H, CH_2-oxepin), 7.49-7.57 (m, 5H, Ar-H), 7.59(s, 1H, CH), 8.55 (s, 1H, Ar-H); MS m/z (%): 340 [M^+] (14), corresponding to the molecular formula $C_{17}H_{12}N_2O_6$ and base peak at 218 (100).

4-(4-Methylbenzylidene)-3,4-dihydro-7-nitrobenzo-[*b*]oxepin-5(2*H*)-one (3e): Yield 0.69 g (42%), m.p. 159-161°C (EtOH); IR (KBr, cm^{-1}): 1667 (C=O); ^{1}H NMR (DMSO-d_6) δ: 2.38 (s, 3H, CH_3), 3.17-3.18 (t, 2H, CH_2-oxepin), 4.51-4.53 (t, 2H, CH_2-oxepin), 7.47-7.51 (m, 5H, Ar-H), 7.52 (s, 1H, CH), 8.31 (d, 1H, Ar-H), 8.55 (s, 1H, Ar-H); MS m/z (%): 309 [M^+] (32), corresponding to the molecular formula $C_{18}H_{15}NO_4$ and base peak at 310 (100).

4-(4-Isopropylbenzylidene)-3,4-dihydro-7-nitrobenzo-[*b*]oxepin-5(2*H*)-one (3f): Yield 0.69 g (42%), m.p. 129-130°C (EtOH); IR (KBr, cm^{-1}): 1661 (C=O); ^{1}H NMR (DMSO-d_6) δ: 1.33 (s, 6H, $2CH_3$), 3.13-3.14 (t, 2H, CH_2-oxepin), 3.22 (m, 1H, CH), 4.48-4.51 (t, 2H, CH_2-oxepin), 7.43-7.47 (m, 5H, Ar-H), 7.51 (s, 1H, CH), 8.32 (d, 1H, Ar-H), 8.54 (s, 1H, Ar-H); MS m/z (%): 337 [M^+] (67), corresponding to the molecular formula $C_{20}H_{19}NO_4$ and base peak at 294 (100).

4-(3,4,5-Trimethoxybenzylidene)-3,4-dihydro-7-nitrobenzo [*b*]oxepin-5(2*H*)-one (3g): Yield 1.43 g (74%), m.p. 201-203°C (EtOH); IR (KBr, cm^{-1}): 1660 (C=O);

^{1}H NMR (DMSO-d_6) δ: 3.17-3.18 (t, 2H, CH_2-oxepin), 3.67 (s, 9H, $3CH_3$), 4.51-4.53 (t, 2H, CH_2-oxepin), 7.17-7.18 (m, 3H, Ar-H), 7.51 (s, 1H, CH), 8.32 (d, 1H, Ar-H), 8.54 (s, 1H, Ar-H); MS m/z (%): 385 [M^+] (12), corresponding to the molecular formula $C_{20}H_{19}NO_7$ and base peak at 386 (100).

3-Aryl-9-nitro-3,3a,4,5-tetrahydro-2*H*-benzo-[*b*]oxepino[4,5-*c*]pyrazole (4a-c)

General Method: To a mixture of compound 3 (2 mM) [3a (0.63 g), 3e (0.62 g), 3f (0.67 g)] and hydrazine hydrate (0.4 ml, 12 mM, 99%) in ethanol (25 ml), few drops of triethylamine were added as a catalyst. The reaction mixture was refluxed for 6h, left to cool to room temperature, poured onto ice-water. The formed solid was collected by filtration, dried and crystallized from the proper solvent to give 4.

3-(4-Fluorophenyl)-9-nitro-3,3a,4,5-tetrahydro-2*H*-benzo[*b*]-oxepino[4,5-*c*]pyrazole (4a): Yield 0.49 g (75%), m.p. 179-181°C (dioxane); IR (KBr, cm^{-1}): 3331 (NH), 1659 (C=N), 1595 and 1350 (NO_2); ^{1}H NMR ($CDCl_3$) δ: 1.89-2.35 (m, 3H, CH_2-oxepin and H_a), 3.17 (t, 2H, CH_2-oxepin), 3.25 (d, 1H, H_b), 6.87-7.17 (m, 6H, Ar-H and NH), 8.1 (d, 1H, Ar-H), 8.4 (s, 1H, Ar-H); MS m/z (%): 327 [M^+] (12), corresponding to the molecular formula $C_{17}H_{14}FN_3O_3$ and base peak at 328 (100).

3-(4-Tolyl)-9-nitro-3,3a,4,5-tetrahydro-2*H*-benzo[*b*]-oxepino [4,5-*c*]pyrazole (4b): Yield 0.46 g (71%), m.p. 139-141°C (EtOH); IR (KBr, cm^{-1}): 3350 (NH), 1658 (C=N), 1598 and 1350 (NO_2); ^{1}H NMR ($CDCl_3$) δ: 1.8-2.3 (m, 3H, CH_2 oxepin and H_a), 2.39 (s, 3H, CH_3), 3.15 (t, 2H, CH_2-oxepin), 3.24 (d, 1H, H_b), 6.9-7.21 (m, 6H, Ar-H and NH), 8.0 (d, 1H, Ar-H), 8.3 (s, 1H, Ar-H); MS m/z (%): 323 [M^+] (35), corresponding to the molecular formula $C_{18}H_{17}N_3O_3$ and base peak at 232 (100).

3-(4-Isopropylphenyl)-9-nitro-3,3a,4,5-tetrahydro-2*H*-benzo[*b*]oxepino[4,5-*c*]pyrazole (4c): Yield 0.50 g (72%), m.p. 145-147°C (MeOH); IR (KBr, cm^{-1}): 3290 (NH), 1650 (C=N), 1582 and 1350 (NO_2); ^{1}H NMR ($CDCl_3$) δ: 1.59 (d, 6H, CH_3),1.89-2.35 (m, 3H, CH_2-oxepin and H_a), 2.60 (m, 1H, CH), 3.17 (t, 2H, CH_2-oxepin), 3.25 (d, 1H, H_b), 6.87-7.17 (m, 6H, Ar-H and NH), 8.1 (d, 1H, Ar-H), 8.4 (s, 1H, Ar-H); MS m/z (%): 351 [M^+] (20), corresponding to the molecular formula $C_{20}H_{21}N_3O_3$ and base peak at 236 (100).

2-Acetyl-3-aryl-9-nitro-3,3a,4,5-tetrahydro-benzo[*b*]-oxepino[4,5-*c*]pyrazole (5a-c)

General Method: A mixture of compound 3 (2 mM) [3a (0.63 g), 3e (0.62 g), 3f (0.67 g)] and hydrazine hydrate (0.4 ml, 12 mM, 99%) in glacial acetic acid (10 ml) was refluxed for 4h. After cooling the formed solid was collected by filtration, dried and crystallized from the proper solvents to give 5.

2-Acetyl-3-(4-fluorophenyl)-9-nitro-3,3a,4,5-tetrahydro-benzo[*b*]oxepino[4,5-*c*]pyrazole (5a): Yield 0.49 g (67%), m.p. 185-188°C (MeOH); IR (KBr, cm^{-1}): 1720 (C=O), 1660 (C=N), 1599 and 1335 (NO_2); ^{1}H NMR ($CDCl_3$) δ: 1.95 (s, 3H, $COCH_3$), 1.99-2.40 (m, 3H, CH_2 oxepin and H_a), 3.26 (t, 2H, CH_2-oxepin), 3.40 (d, 1H, H_b), 6.8-7.1 (m, 5H, Ar-H), 8.1 (d, 1H, Ar-H), 8.42 (s, 1H, Ar-H); MS m/z (%): 369 [M^+] (53), corresponding to the molecular formula $C_{19}H_{16}FN_3O_4$ and base peak at 274 (100).

2-Acetyl-3-(4-tolyl)-9-nitro-3,3a,4,5-tetrahydro-benzo-[*b*]oxepino[4,5-*c*]pyrazole (5b): Yield 0.50 g (68%), m.p. 163-165°C (EtOH); IR (KBr, cm^{-1}): 1725 (C=O), 1665 (C=N), 1601 and 1332 (NO_2); ^{1}H NMR ($CDCl_3$) δ: 1.9 (s, 3H, $COCH_3$), 1.95-2.35 (m, 3H, CH_2 oxepin and H_a), 2.40 (s, 3H, CH_3), 3.26 (t, 2H, CH_2 oxepin), 3.36 (d, 1H, H_b), 6.9-7.1 (m, 5H, Ar-H), 8.2 (d, 1H, Ar-H), 8.45 (s, 1H, Ar-H); MS m/z (%): 365 [M^+] (33), corresponding to the molecular formula $C_{20}H_{19}N_3O_4$ and base peak at 350 (100).

2-Acetyl-3-(4-isopropylphenyl)-9-nitro-3,3a,4,5-tetrahydro-benzo[*b*]oxepino[4,5-*c*]pyrazole (5c): Yield 0.53 g (68%), m.p. 178-181°C (2-PrOH); IR (KBr, cm^{-1}): 1720 (C=O), 1655 (C=N), 1590 and 1329 (NO_2); ^{1}H NMR ($CDCl_3$) δ: 1.58 (d, 6H, CH_3), 1.95 (s, 3H, $COCH_3$), 1.99-2.40 (m, 3H, CH_2 oxepin and H_a), 2.59 (m, 1H, CH), 3.26 (t, 2H, CH_2oxepin), 3.40 (d, 1H, H_b), 6.8-7.1 (m, 5H, Ar-H), 8.1 (d, 1H, Ar-H), 8.42 (s, 1H, Ar-H); MS m/z (%): 393 [M^+] (25), corresponding to the molecular formula $C_{22}H_{23}N_3O_4$ and base peak at 250 (100).

3-Aryl-9-nitro-2-phenyl-3,3a,4,5-tetrahydro-benzo-[*b*]oxepino[4,5-*c*]pyrazole (6a-d)

General Method: A mixture of compound 3 (2 mM) [3a (0.63 g), 3c (0.75 g), 3e (0.62 g), 3f (0.67 g)] and phenylhydrazine (1.21 ml, 12 mM, 99%) in glacial acetic acid (10 ml), was refluxed for 6h, cooled and poured onto ice-water containing 5% sulphuric acid (1 ml), the solid formed was collected by filtration, dried and crystallized from the proper solvents to give 6.

3-(4-Fluorophenyl)-9-nitro-2-phenyl-3,3a,4,5-tetrahydro-benzo[*b*]oxepino[4,5-*c*]pyrazole (6a): Yield 0.64 g (79%), m.p. 210-215°C (AcOH); IR (KBr, cm^{-1}): 1665 (C=N), 1595 and 1350 (NO_2); ^{1}H NMR ($CDCl_3$) δ: 1.94-2.35 (m, 3H, CH_2 oxepin and H_a), 3.22 (t, 2H, CH_2oxepin), 3.48 (d, 1H, H_b), 7.1-7.7 (m, 10H, Ar-H), 8.1 (d, 1H, Ar-H), 8.5 (s, 1H, Ar-

H); MS m/z (%): 403 [M^+] (12), corresponding to the molecular formula $C_{23}H_{18}FN_3O_3$ and base peak at 231 (100).

3-(4-Bromophenyl)-9-nitro-2-phenyl-3,3a,4,5-tetrahydro-benzo[*b*]oxepino[4,5-*c*]pyrazole (6b): Yield 0.70 g (75%), m.p. 208-210°C (AcOH); IR (KBr, cm^{-1}): 1661 (C=N), 1597 and 1343 (NO_2); ^{1}H NMR (DMSO-d_6) δ: 1.89-2.32 (m, 3H, CH_2 oxepin and H_a), 3. 20 (t, 2H, CH_2 oxepin), 3.43 (d, 1H, H_b), 6.9-8.0 (m, 10H, Ar-H), 8.2 (d, 1H, Ar-H), 8.5 (s, 1H, Ar-H); MS m/z (%): 464 [M^+] (42), corresponding to the molecular formula $C_{23}H_{18}BrN_3O_3$ and base peak at 308 (100).

3-(4-Tolyl)-9-nitro-2-phenyl-3,3a,4,5-tetrahydro-benzo-[*b*]oxepino[4,5-*c*]pyrazole (6c): Yield 0.62 g (78%), m.p. 190-195°C (acetic acid); IR (KBr, cm^{-1}): 1668 (C=N), 1598 and 1355 (NO_2); ^{1}H NMR (DMSO-d_6) δ: 1.91-2.34 (m, 3H, CH_2 oxepin and H_a), 2.36 (s, 3H, CH_3), 3.21 (t, 2H, CH_2 oxepin), 3.53 (d, 1H, H_b), 7.2-7.8 (m, 10H, Ar-H), 8.2 (d, 1H, Ar-H), 8.5 (s, 1H, Ar-H); MS m/z (%): 399 [M^+] (12), corresponding to the molecular formula $C_{24}H_{21}N_3O_3$ and base peak at 232 (100).

3-(4-Isopropylphenyl)-9-nitro-2-phenyl-3,3a,4,5-tetrahydro-benzo[*b*]oxepino[4,5-*c*]pyrazole (6d): Yield 0.62 g (73%), m.p. 205-209°C (AcOH); IR (KBr, cm^{-1}): 1651 (C=N), 1550 and 1350 (NO_2); ^{1}H NMR ($CDCl_3$) δ: 1.61 (d, 6H, CH_3),1.94-2.35 (m, 3H, CH_2 oxepin and H_a), 2.59 (m, 1H, CH), 3.22 (t, 2H, CH_2 oxepin), 3.48 (d, 1H, H_b), 7.1-7.7 (m, 10H, Ar-H), 8.1 (d, 1H, Ar-H), 8.5 (s, 1H, Ar-H); MS m/z (%): 427 [M^+] (10), corresponding to the molecular formula $C_{26}H_{25}N_3O_3$ and base peak at 397 (100).

4-Aryl-10-nitro-1,2,5,6-tetrahydro-2-thioxobenzo-[*b*]oxepino[4,5-*b*]pyridine-3-carbonitrile (7a,b)

General Method: A mixture of compound 3 (2 mM) [3b (0.66 g), 3e (0.62 g)] and cyanothioacetamide (0.21 g, 2 mM) in sodium methoxide solution (0.025 g sodium/7.5 ml methanol) was refluxed 3h. The reaction mixture was cooled, poured onto ice-water containing 5% hydrochloric acid; the solid formed was collected by filtration, dried and crystallized from the proper solvent to give 7.

4-(4-Chlorophenyl)-10-nitro-1,2,5,6-tetrahydro-2-thioxobenzo[*b*]oxepino[4,5-*b*]pyridine-3-carbonitrile (7a): Yield 0.53 g (65%), m.p. 179-181°C (dioxane);IR (KBr, cm^{-1}): 3325 (NH), 2225 (CN); ^{1}H NMR ($CDCl_3$) δ 2.48 (t, 2H, CH_2-oxepin), 3.23 (t, 2H, CH_2-oxepin), 6.91-7.95 (m, 5H, Ar-H), 8.0 (d, 1H, Ar-H), 8.2 (s, 1H, Ar-H), 9.45 (br. s, 1H, NH, exchangeable with D_2O); MS m/z (%): 409 [M^+] (15), corresponding to the molecular formula $C_{20}H_{12}ClN_3O_3S$ and base peak at 383 (100).

4-(4-Tolyl)-10-nitro-1,2,5,6-tetrahydro-2-thioxobenzo-[*b*]oxepino[4,5-*b*]pyridine-3-carbonitrile (7b): Yield 0.53 g (68%), m.p. 230-233°C (2-PrOH); IR (KBr, cm^{-1}): 3330 (NH), 2220 (CN); ^{1}H NMR ($CDCl_3$) δ 2.34 (s, 3H, CH_3), 2.51 (t, 2H, CH_2-oxepin), 3.25 (t, 2H, CH_2-oxepin), 7.08-7.8 (m, 5H, Ar-H), 7.9 (d, 1H, Ar-H), 8.1 (s, 1H, Ar-H), 9.43 (br. s, 1H, NH, exchangeable with D_2O); MS m/z (%): 389 [M^+] (30), corresponding to the molecular formula $C_{21}H_{15}N_3O_3S$ and base peak at 363 (100).

4-Aryl-10-nitro-3,4,5,6-tetrahydro-benzo[*b*]oxepino[4,5-*d*]pyrimidine-2(1*H*)-imine (8a-d)

General Method: A mixture of compound 3 (2 mM) [3a (0.63 g), 3b (0.66 g), 3d (0.68 g), 3e (0.62 g)] and guanidine hydrochloride (0.19 g, 2 mM) was added to a solution of ethanol absolute (7.5 ml) containing 0.50 g NaOH. The r eaction mixture was refluxed for 4h, cooled, poured gradually with stirring onto cold water. The solid formed was collected by filtration, washed with water and crystallized from the proper solvent to give 8.

4-(4-Fluorophenyl)-10-nitro-3,4,5,6-tetrahydro-benzo-[*b*]oxepino[4,5-*d*]pyrimidine-2(1*H*)-imine (8a): Yield 0.44 g (62%), m.p. 242-245°C (dioxane); IR (KBr, cm^{-1}): 3450, 3300 and 3150 (NH), 1568 and 1350 (NO_2); ^{1}H NMR (DMSO-d_6) δ: 2.48 (t, 2H, CH_2-oxepin), 3.20 (t, 2H, CH_2-oxepin), 5.01 (s, 1H, pyrimidine-H), 6.85-7.30 (m, 5H, Ar-H), 8.11 (d, 1H, Ar-H), 8.3 (s, 1H, Ar-H), 8.79, 9.08, 10.05 (s, 3H, 3NH, exchangeable with D_2O); MS m/z (%): 354 [M^+] (43), corresponding to the molecular formula $C_{18}H_{15}FN_4O_3$ and base peak at 259 (100).

4-(4-Chlorophenyl)-10-nitro-3,4,5,6-tetrahydro-benzo-[*b*]oxepino[4,5-*d*]pyrimidine-2(1*H*)-imine (8b): Yield 0.42 g (57%), m.p. 273-275°C (dioxane); IR (KBr, cm^{-1}): 3455, 3350 and 3225 (NH), 1601 and 1350 (NO_2); ^{1}H NMR (DMSO-d_6) δ: 2.50 (t, 2H, CH_2-oxepin), 3.22 (t, 2H, CH_2-oxepin), 5.1 (s, 1H, pyrimidine-H), 7.11-7.40 (m, 5H, Ar-H), 8.1 (d, 1H, Ar-H), 8.3 (s, 1H, Ar-H), 8.81, 9.12, 10.13 (s, 3H, 3NH, exchangeable with D_2O); MS m/z (%): 371 [M^+] (61), corresponding to the molecular formula $C_{18}H_{15}ClN_4O_3$ and base peak at 335 (100).

4-(2-Nitrophenyl)-10-nitro-3,4,5,6-tetrahydro-benzo-[*b*]oxepino[4,5-*d*]pyrimidine-2(1*H*)-imine (8c): Yield 0.52 g (68%), m.p. 263-265°C (EtOH); IR (KBr, cm^{-1}): 3450, 3365 and 3315 (NH); ^{1}H NMR (DMSO-d_6) δ: 2.46 (t, 2H, CH_2-oxepin), 3.18 (t, 2H, CH_2-oxepin), 4.9 (s, 1H, pyrimidine-H), 7.08-8.1 (m, 5H, Ar-H), 8.25 (d, 1H, Ar-H),

8.34 (s, 1H, Ar-H), 8.6, 9.1, 10.2 (s, 3H, 3NH, exchangeable with D_2O); MS m/z (%): 382 [M^++1] (50), corresponding to the molecular formula $C_{18}H_{15}N_5O_5$ and base peak at 366 (100).

4-(4-Tolyl)-10-nitro-3,4,5,6-tetrahydro-benzo[*b*]oxepino-[4,5-*d*]pyrimidine-2(1*H*)-imine (8d): Yield 0.51 g (73%), m.p. 231-238°C (dioxane); IR (KBr, cm^{-1}): 3162, 3315 and 3460 (NH); ^{1}H NMR (DMSO-d_6) δ: 2.38 (s, 3H, CH_3), 2.49 (t, 2H, CH_2-oxepin), 3.21 (t, 2H, CH_2-oxepin), 5.09 (s, 1H, pyrimidine-H), 7.0-7.2 (m, 5H, Ar-H), 8.1 (d, 1H, Ar-H), 8.2-8.3 (s, 1H, Ar-H), 8.9, 9.2, 10.2 (s, 3H, 3NH, exchangeable with D_2O); MS m/z (%): 350 [M^+] (20), corresponding to the molecular formula $C_{19}H_{18}N_4O_3$ and base peak at 335 (100).

4-Aryl-10-nitro-3,4,5,6-tetrahydro-benzo[*b*]oxepino[4,5-*d*]pyrimidine-2(1*H*)-thione (9a-d)

General Method: To a mixture of 3 (5 mM) [3b (1.65 g), 3e (1.55 g), 3f (1.68 g), 3g (1.92 g)] with thiourea (0.5 g, 6.5 mM) in ethanol (25 ml), was added a solution of potassium hydroxide (0.5g/6 ml H_2O). The reaction mixture was refluxed for 4h, poured onto water. The solid formed was collected by filtration, washed with water, dried and crystallized form the appropriate solvent to give 9.

4-(4-Chlorophenyl)-10-nitro-3,4,5,6-tetrahydro-benzo-[*b*]oxepino[4,5-*d*]pyrimidine-2(1*H*)-thione (9a): Yield 1.53 g (79%), m.p. 246-248°C (dioxane); IR (KBr, cm^{-1}): 3420 and 3370 (NH); ^{1}H NMR (DMSO-d_6) δ: 2.49 (t, 2H, CH_2-oxepin), 3.20 (t, 2H, CH_2-oxepin), 4.95 (s, 1H, pyrimidine-H), 6.9-7.4 (m, 5H, Ar-H), 8.1 (d, 1H, Ar-H), 8.2-8.3 (s, 1H, Ar-H), 9.2, 10.2 (s, 2H, 2NH, exchangeable with D_2O); MS m/z (%): 388 [M^+] (67), corresponding to the molecular formula $C_{18}H_{14}ClN_3O_3S$ and base peak at 276 (100).

4-(4-Tolyl)-10-nitro-3,4,5,6-tetrahydro-benzo[*b*]-oxepino[4,5-*d*]pyrimidine-2(1*H*)-thione (9b): Yield 1.43 g (78%), m.p. 219-221°C (AcOH); IR (KBr, cm^{-1}): 3425 and 3380 (NH); ^{1}H NMR ($CDCl_3$) δ: 2.39 (s, 3H, CH_3), 2.50 (t, 2H, CH_2-oxepin), 3.22 (t, 2H, CH_2-oxepin), 4.9 (s, 1H, pyrimidine-H), 7.02-7.2 (m, 5H, Ar-H), 7.9 (d, 1H, Ar-H), 8.2 (s, 1H, Ar-H), 9.3, 10.4 (s, 2H, NH, exchangeable with D_2O); MS m/z (%): 367 [M^+] (51), corresponding to the molecular formula $C_{19}H_{17}N_3O_3S$ and base peak at 252 (100).

4-(4-Isopropylphenyl)-10-nitro-3,4,5,6-tetrahydro-benzo-[*b*]oxepino[4,5-*d*]pyrimidine-2(1*H*)-thione (9c): Yield 1.48 g (75%), m.p. 230-232°C (AcOH); IR (KBr, cm^{-1}): 3410 and 3362 (NH); ^{1}H NMR (DMSO-d_6) δ: 1.62 (d, 6H, CH_3), 2.48 (t, 2H, CH_2-oxepin), 2.63 (m, 1H, CH) , 3.20 (t, 2H, CH_2-oxepin), 4.90 (s, 1H, pyrimidine-H), 6.9-7.4 (m, 5H, Ar-H), 8.1 (d, 1H, Ar-H), 8.3 (s, 1H, Ar-H), 9.2, 10.2 (s, 2H, 2NH, exchangeable with D_2O); MS m/z (%): 395 [M^+] (25), corresponding to the molecular formula $C_{21}H_{21}N_3O_3S$ and base peak at 352 (100).

4-(3,4,5-Trimethoxyphenyl)-10-nitro-3,4,5,6-tetrahydro-benzo[*b*]oxepino[4,5-*d*]pyrimidine-2(1*H*)-thione (9d): Yield 1.53 g (71%), m.p. 250-251°C (AcOH); IR (KBr, cm^{-1}): 3432 and 3389 (NH); ^{1}H NMR (DMSO-d_6) δ: 2.49 (t, 2H, CH_2-oxepin), 3.01 (s, 9H, CH_3), 3.22 (t, 2H, CH_2-oxepin), 4.95 (s, 1H, pyrimidine-H), 6.7-7.48 (m, 3H, Ar-H), 8.1 (d, 1H, Ar-H), 8.3 (s, 1H, Ar-H), 9.32, 10.38 (s, 2H, 2NH, exchangeable with D_2O); MS m/z (%): 443 [M^+] (12), corresponding to the molecular formula $C_{21}H_{21}N_3O_6S$ and base peak at 444 (100).

5-Aryl-2,3,5,6-tetrahydro-11-nitrobenzo[*b*]oxepino[5,4-*d*]thiazolo[3,2-*a*]pyrimidine-3(5*H*)-one (10a,b)

General Method: A mixture of 9 (2 mM) [9a (0.78g), 9b (0.73 g)], chloroacetic acid (0.2 g, 2.2 mM), fused sodium acetate (1.25 g, 15 mM), acetic acid (10 ml) and acetic anhydride (5 ml) was refluxed for 4h, cooled and poured onto water. The solid formed was collected by filtration, washed with water, dried and crystallized form ethanol to give 10.

5-(4-Chlorophenyl)-2,3,5,6-tetrahydro-11-nitrobenzo-[*b*]oxepino[5,4-*d*]thiazolo[3,2-*a*]pyrimidine-3(5*H*)-one (10a): Yield 1.39 g (65%), m.p. 200-206°C (2-PrOH); IR (KBr, cm^{-1}): 1734 (C=O); ^{1}H NMR (DMSO-d_6) δ: 2.46 (t, 2H, CH_2-oxepin), 3.2 (s, 2H, thiazole-H), 3.23 (t, 2H, CH_2-oxepin), 5.7 (s, 1H, pyrimidine-H), 7.2-7.8 (m, 5H, Ar-H), 7.9 (d, 1H, Ar-H), 8.1 (s, 1H, Ar-H); MS m/z (%): 429 [M^++2] (25), corresponding to the molecular formula $C_{20}H_{14}ClN_3O_4$ and base peak at 316 (100).

5-(4-Tolyl)-2,3,5,6-tetrahydro-11-nitrobenzo[*b*]-oxepino[5,4-*d*]thiazolo[3,2-*a*]pyrimidine-3(5*H*)-one (10b) Yield 1.28 g (63%), m.p. 186-190°C (EtOH); IR (KBr, cm^{-1}): 1734 (C=O); ^{1}H NMR (DMSO-d_6) δ: 2.35 (s, 3H, CH_3), 2.48 (t, 2H, CH_2-oxepin), 3.05 (s, 2H, thiazole-H), 3.22 (t, 2H, CH_2-oxepin), 5.5 (s, 1H, pyrimidine-H), 7.31-7.8 (m, 5H, Ar-H), 7.9 (d, 1H, Ar-H), 8.1 (s, 1H, Ar-H); MS m/z (%): 409 [M^++2] (14), corresponding to the molecular formula $C_{21}H_{17}N_3O_4S$ and base peak at 392 (100).

5-Aryl-2-arylmethylene-6,7-dihydro-11-nitrobenzo[*b*]-oxepino[5,4-*d*]thiazolo[3,2-*a*]pyrimidine-3(5*H*)-one (11a,b)

Method A: A mixture of equimolar amounts (2 mM) of compound 10 [10a (0.86 g), 10b (0.82 g)] and appropriate aromatic aldehyde [chlorobenzaldehyde (0.28 g), 4-tolualdehyde (0.25 g)], glacial acetic acid (10 ml), acetic anhydride (5 ml) and fused sodium acetate (0.5 g, 6 mM) was refluxed for 2h, cooled and poured onto cold water. The solid formed was collected by filtration and crystallized from proper solvent to afford 11.

Method B: A mixture of 9 (2 mM) [9a (0.78 g), 9b (0.73 g)], chloroacetic acid (5 ml) and a proper aromatic aldehyde (2 mM) [chlorobenzaldehyde (0.28 g), 4-tolualdehyde (0.25 g)], was refluxed for 4h. Then the reaction mixture was cooled and poured onto cold water. The solid formed was collected by filtration, dried and crystallized from the proper solvent to give 11 as identified by the TLC, mp and mixed mp with authentic samples from Method A.

5-(4-Chlorophenyl)-2-(4-methylbenzylidene)-6,7-dihydro-11-nitrobenzo[*b*]oxepino[5,4-*d*]thiazolo[3,2-*a*]pyrimidine-3(5*H*)-one (11a): Yield 0.59 g (55%) (A), 0.65 g (61%) (B), m.p. 233-237°C (AcOH); IR (KBr, cm^{-1}): 1703 (C=O); ^{1}H NMR (DMSO-d_6) δ: 2.39 (s, 3H, CH_3), 2.46 (t, 2H, CH_2-oxepin), 3.20 (t, 2H, CH_2-oxepin), 5.8 (s, 1H, pyrimidine-H), 6.98 (s, 1H, CH), 7.1-7.8 (m, 9H, Ar-H), 7.79-8.1 (d, 1H, Ar-H), 8.20 (s, 1H, Ar-H); MS m/z (%): 530 [M^++1] (22), corresponding to the molecular formula $C_{28}H_{20}ClN_3O_4S$ and base peak at 438 (100).

5-(4-Tolyl)-2-(4-chlorobenzylidene)-6,7-dihydro-11-nitrobenzo[*b*]oxepino[5,4-*d*]thiazolo[3,2-*a*]pyrimidine-3(5*H*)-one (11b): Yield 0.61 g (57%) (A), 0.73 g (69%) (B), m.p. 245-250°C (AcOH); IR (KBr, cm^{-1}): 1703 (C=O); ^{1}H NMR (DMSO-d_6) δ: 2.40 (s, 3H, CH_3), 2.50 (t, 2H, CH_2-oxepin), 3.22 (t, 2H, CH_2-oxepin), 6.1 (s, 1H, pyrimidine-H), 6.96 (s, 1H, CH), 7.1-7.8 (m, 9H, Ar-H), 7.79-8.1 (d, 1H, Ar-H), 8.21 (s, 1H, Ar-H); MS m/z (%): 530 [M^++1] (20), corresponding to the molecular formula $C_{28}H_{20}ClN_3O_4S$ and base peak at 404 (100).

Pharmacological Screening

Anticancer Activity: The selected compounds, within DTP Human Tumor Cell Line Screen by the National Cancer Institute (NCI), were evaluated *in vitro* against the full panel of NCI's 60 cancer cell lines derived from nine cancer cell types, including different cancers of the non-small cell lung (A549/ATCC; EKVX; HOP-62; NCI-H226; NCI-H23; NCI-H460; NCI-H522), colon (COLO 205; HCC-2998; HCT-116; HCT-15; HT29; KM12; SW-620), breast (HS 578T; MCF7; MDA-MB-231/ATCC; MDA-MB-435; MDA-MB-468; NCI/ADR-RES; T-47D), ovary (IGROV1; OVCAR-3; OVCAR-4; OVCAR-5; OVCAR-8; SK-OV-3), renal (786-0; A498; ACHN; CAKI-1; RXF 393; SN12C; UO-31), prostate (PC-3, DU-145), CNS (SF-268; SF-295; SF-539; SNB-19; SNB-75; U251) and human tumor cell lines, leukemia (CCRF-CEM; HL-60(TB); K-562; MOLT-4; RPMI-8226; SR), melanoma (LOX IMIV; M14; SK-MEL-2; SK-MEL-28; SK-MEL-5; UACC-257; UACC-62). In this protocol, each cell line is inoculated and preincubated on a microtiter plate. Test agents are then added at a single concentration (10^{-4} M) and the culture incubated for 48h. End point determinations are made with alamar blue REF. Results for each test agent are reported as the percent of growth of the treated cells when compared to the untreated control cells (Table 1). Details of this evaluation method and the complementary information related with the activity pattern over all cell lines, have been published [18-21].

Anti-inflammatory Activity

Animals: All animals were obtained from the animal house colony of the National Research Center, Cairo, Egypt. All animals were allowed free access to water and were kept on a constant standard diet. All procedures involving animals were carried out in accordance with the guide for the care and use of laboratory animals (National Academy of Science of Egypt) and were approved by the Animals Studies Committee at Washington University. Adult male albino rats, weighing 150-180 g, fasted for 12h and used for determining the anti-inflammatory activity and LD_{50}.

Determination of Acute Toxicity (LD_{50}): The test compounds were administered orally at different dose levels in separate groups of animals. After 24h of drug administration percent mortality in each group was observed. From the data obtained, the lethal dose (LD_{50}) was calculated by the method of Smith [22].

Carrageenan-induced Edema (Rats Paw Test): The inhibitory activity of the studied compounds on carrageenan-induced rat's paw edema was carried out according to the method of Winter *et al*., [23, 24]. Groups of rats, each of 8 animals were orally dosed with the test compounds at a dose level of 2.5 and 5 mg/kg an hour before carrageenan challenge. Foot paw edema was induced by sub planter injection of 0.05 ml of 1% suspension of carrageenin in saline into the planter tissue of one hind paw. An equal volume of saline was injected to the other hind paw and served as control. Four hours after drug administration the animal was decapitated,

Table 1: *In vitro* results expressed as growth inhibition of cancer cell lines of the tested derivatives [GI_{50} (iM)]*

Panel	Cell line	3a	3g	4a	7a	8b	8c	9a	10	11a
Non-Small Cell Lung Cancer	A549/ATCC	7.42	2.28	NA	NA	3.46	5.28	NA	NA	NA
	EKVX	2.94	NA	NA	9.74	8.94	NA	15.49	NA	NA
	HOP-62	NA	9.72	7.13	5.73	3.24	NA	5.43	3.72	NA
	NCI-H226	11.93	NA	8.62	18.72	17.60	8.24	NA	NA	3.42
	NCI-H23	25.08	NA	NA	NA	4.94	5.17	NA	NA	NA
	NCI-H460	2.82	9.55	NA	6.12	4.32	NA	1.68	NA	NA
	NCI-H522	23.31	23.64	3.31	34.74	27.44	19.26	32.85	NA	21.96
Colon Cancer	COLO 205	NA	NA	NA	16.54	NA	NA	NA	3.46	NA
	HCC-2998	NA	NA	NA	NA	NA	NA	NA	7.94	NA
	HCT-116	1.94	4.40	1.13	4.40	8.49	0.90	7.44	2.26	NA
	HCT-15	NA	NA	5.83	NA	NA	NA	NA	16.70	NA
	HT29	NA	1.84	2.43	NA	NA	NA	NA	3.96	NA
	KM12	NA	NA	NA	NA	NA	NA	2.35	5.43	NA
	SW-620	8.47	5.78	NA	NA	6.32	NA	NA	12.66	NA
Breast Cancer	HS 578T	1.58	6.48	NA	NA	NA	NA	15.36	NA	11.72
	MCF7	7.55	11.56	2.92	NA	1.84	9.58	NA	NA	5.42
	MDA-MB-231/ATCC	NA	9.72	2.40	NA	1.00	NA	8.18	3.50	7.32
	MDA-MB-435	1.56	NA	NA	NA	2.54	3.45	NA	NA	NA
	MDA-MB-468	NA	3.94	1.69	NA	NA	1.82	5.52	NA	NA
	NCI/ADR-RES	NA	NA	NA	NA	NA	NA	NA	NA	NA
	T-47D	NA	NA	NA	NA	2.27	1.92	5.46	NA	8.46
Ovarian Cancer	IGROV1	NA	NA	11.35	NA	NA	NA	NA	NA	NA
	OVCAR-3	5.76	18.94	NA	28.81	4.54	NA	16.98	22.57	16.84
	OVCAR-4	6.54	4.52	NA	NA	NA	NA	4.36	NA	NA
	OVCAR-5	NA	NA	NA	1.92	9.82	10.62	NA	NA	NA
	OVCAR-8	NA	5.32	NA	NA	NA	NA	2.18	NA	NA
	SK-OV-3	NA	NA	NA	12.62	NA	NA	NA	NA	NA
Renal Cancer	786-0	14.23	NA	NA	NA	8.74	6.68	NA	NA	NA
	A498	6.68	NA	NA	NA	4.36	7.87	13.40	7.50	2.91
	ACHN	18.90	35.28	1.91	9.68	3.51	3.41	8.79	28.10	NA
	CAKI-1	NA	17.32	6.31	20.18	12.65	3.77	15.74	NA	NA
	RXF 393	4.21	9.81	NA	3.25	NA	4.54	4.70	NA	4.26
	SN12C	NA	NA	1.12	NA	3.80	NA	NA	NA	2.18
	UO-31	19.90	11.99	24.87	3.80	4.09	9.16	NA	5.33	10.47
Prostate Cancer	PC-3	NA	3.40	1.82	NA	NA	NA	NA	NA	NA
	DU-145	NA	2.36	NA	NA	1.22	NA	NA	NA	NA
CNS Cancer	SF-268	NA	NA	NA	NA	NA	NA	NA	NA	NA
	SF-295	NA	NA	NA	NA	NA	NA	NA	NA	NA
	SF-539	3.52	3.36	8.07	2.46	2.38	NA	5.82	NA	3.64
	SNB-19	NA	NA	NA	NA	NA	NA	NA	NA	0.94
	SNB-75	9.97	20.92	17.68	12.67	3.86	NA	3.46	1.58	3.92
	U251	2.38	NA	NA	6.37	11.42	4.72	NA	NA	NA
Leukemia	CCRF-CEM	6.80	NA	14.68	3.57	NA	NA	NA	3.17	5.91
	HL-60(TB)	2.68	NA	9.96	9.93	3.52	NA	NA	NA	NA
	K-562	NA	NA	NA	25.90	2.72	NA	7.54	NA	NA
	MOLT-4	7.93	2.98	4.28	15.54	3.52	2.12	NA	NA	5.60
	RPMI-8226	11.92	6.70	13.88	27.56	NA	5.88	2.34	11.90	19.41
	SR	7.93	25.66	NA	30.74	10.64	19.52	NA	NA	NA
Melanoma	LOX IMIV	3.57	8.50	NA	12.94	11.92	3.72	12.49	2.40	3.78
	M14	NA	NA	1.41	2.38	NA	NA	NA	NA	2.70
	SK-MEL-2	4.46	2.54	NA	2.91	2.84	NA	NA	3.90	6.95
	SK-MEL-28	NA	NA	NA	NA	NA	NA	NA	NA	NA
	SK-MEL-5	11.20	7.82	NA	NA	NA	3.98	10.97	NA	3.51
	UACC-257	3.88	NA	3.22	NA	NA	10.89	NA	NA	NA
	UACC-62	9.72	11.65	7.63	15.36	10.98	5.46	2.76	4.76	7.22

*Data obtained from NCI's in vitro disease-oriented tumor cell screen; GI_{50}: drug molar concentration causing 50% cell growth inhibition, NA= No Activity

Table 2: Anti-inflammatory activities of some new synthesized products

Compound	Acute toxicity (ALD_{50} mg kg^{-1} p.o.)	Ulcerogenic activity (UD_{50} mg kg^{-1} i.p.)	Anti-inflammatory activity		
			Dose mg kg^{-1} p.o.	% Inhibition of oedema	% inhibition of plasma PGE_2
3f	1316.84	197.8	2.5	83.69	78.97
			5	87.96	86
3 g	1123.55	206.12	2.5	87.67	82.51
			5	91.22	88.79
4c	1044.18	219.1	2.5	87.86	84.98
			5	93.98	91
5c	1345.78	184.24	2.5	80.85	75.17
			5	85.86	82.39
6d	967.98	131.2	2.5	91.16	86.89
			5	97.98	92.98
7a	798.98	131.89	2.5	94.2	88.17
			5	99.88	93.6
8b	1242.78	206.4	2.5	85.78	81.19
			5	89.67	86.07
8c	2453.96	197.86	2.5	90.99	85.39
			5	96.83	92.08
9c	1709.89	118.78	2.5	92.2	87.47
			5	98.65	93.06
9d	1935.44	149.9	2.5	89.76	84.85
			5	95.97	91.8
Diclofenac Potassium	2345.87	66.7	2.5	77	72
			5	80.12	81.4

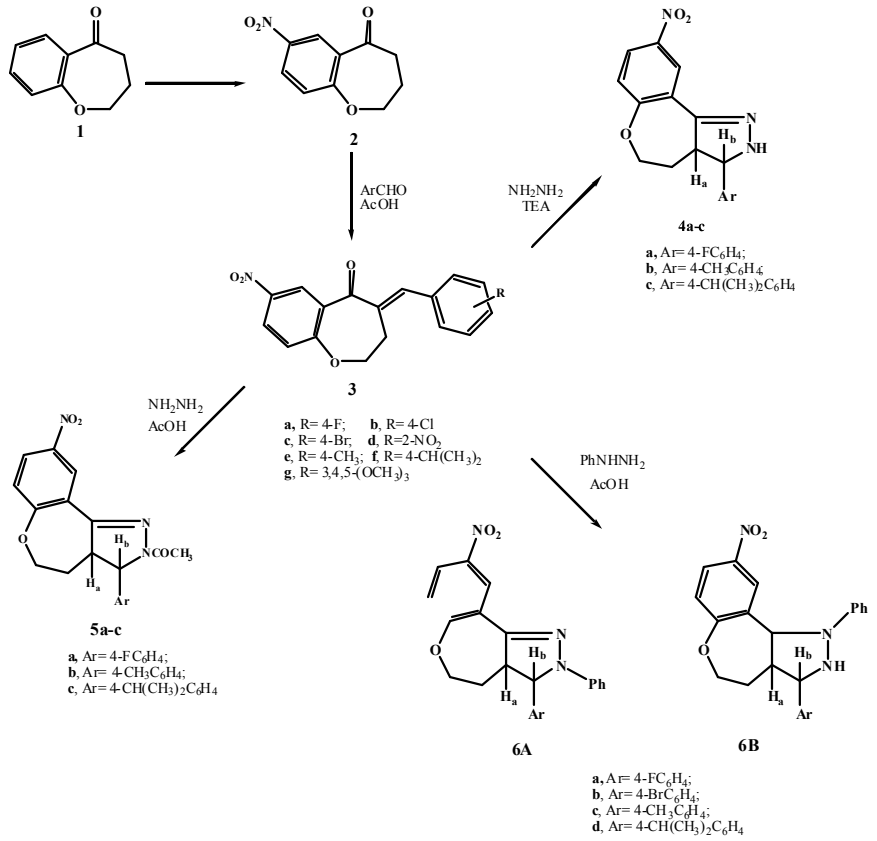
blood was collected and the paws were rapidly excised. The average weight of edema was estimated for the treated as well as the control group and the percentage inhibition of weight of edema was also evaluated; then percentage protection against edema was estimated (Table 2). Diclofenac® (2.5 and 5 mg/kg) was employed as standard reference against which the test compounds were compared.

Estimation of plasma prostaglandin E_2 (PGE_2): Heparinized blood samples were collected from rats (n= 8), plasma was separated by centrifugation at 12 000 × g for 2 min at 4°C and immediately stored frozen -20°C until use. The designs correlate-EIA prostaglandin in E_2 (PGE_2) kit is a competitive immune assay for the quantitative determination of PGE_2 in biological fluids. The kit uses a monoclonal antibody to PGE_2 to bind, in a competitive manner, the PGE_2 in the sample. After a simultaneous incubation at room temperature the excess reagents were washed away and the substrate was added. After a short incubation time the enzyme reaction was stopped and the yellow color generated was read on a micro plate reader (DYNATCH, MR 5000) at 405 nm. The intensity of the bound yellow color is inversely proportional to the concentration of PGE_2 in either standard or samples. The percentage inhibition of plasma PGE_2 for each compound was estimated (Table 2) [25-27].

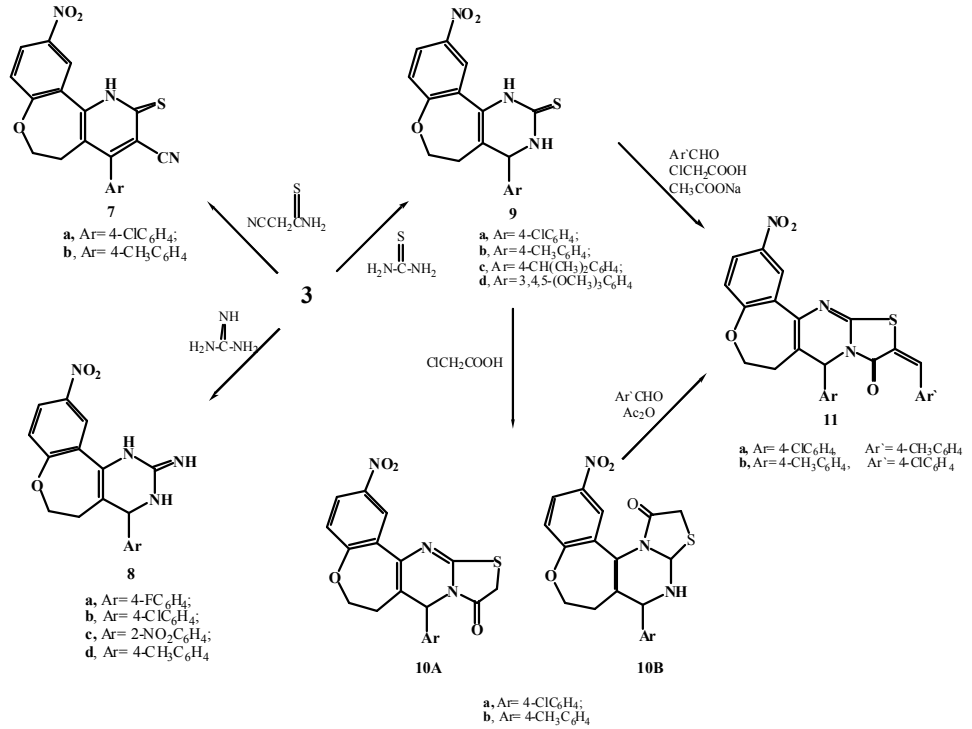
Determination of Ulcerogenic Activity: Ulcerogenic activity was determined according to the method of Verma *et al.*, [28]. In this method, adult albino rats, fasted 24h prior to the administration of drugs, were divided into groups of ten animals each. Water was allowed ad libitum to the animals. The test compounds and standard drugs were given intraperitoneally and the animals sacrificed 8h after drugs treatment. The stomach, duodenum and jejunum were removed and examined with a hand lens for any evidence of (a) shedding of epithelium (b) potential and frank haemorrhage and (c) erosion or discrete ulceration with or without perforation. The presence of any one of these criteria was considered to be an evidence of ulcerogenic activity.

RESULTS AND DISCUSSION

Chemistry: Nitration of compound 1 was performed either by using fuming nitric acid (method A) or a mixture of sulfuric and nitric acids (method B) to get the required starting material 3,4-dihydro-7-nitrobenzo [*b*]oxepin-5(2*H*)-one 2 (Scheme 1). The ketone 2 was reacted with some aromatic aldehydes in the presence of conc. sulphuric acid and glacial acetic acid (1:3) mixture to get the arylmethyelene derivatives 3. The obtained ketone 3 was reacted with hydrazine hydrate in refluxed ethanol affording the benzo[*b*]oxepino[4, 5-*c*]pyrazole



Scheme 1



Scheme 2

derivatives 4. Meanwhile, the reaction of 3 with hydrazine hydrates and phenylhydrazine in glacial acetic acid afforded 2-acetyl- 5 and 2-phenylpyrazole 6 derivatives, respectively. Both IR and ^{1}H NMR spectral data showed the absence of NH group indicating that the formation of the cyclized product 6A is tentatively favored over the isomeric structure 6B.

Condensation of compounds 3 with cyanothioacetamide in methanol, in the presence of a catalytic amount of sodium methoxide, afforded the corresponding pyridinethiones 7 (Scheme 2). Furthermore, the synthesis of novel tricyclic structures containing pyrimidine ring was accomplished by condensation of arylmethylene derivatives 3 with guanidine hydrochloride in the presence of sodium hydroxide to yield aminopyrimidine derivatives 8, meanwhile, the reaction of 3 with thiourea in refluxing ethanol in the presence of potassium hydrooxide as a catalyst afforded the pyrimidinethiones 9.

In the second set of the synthetic experiments shown in Scheme 2 the thioxopyrimidine 9 was reacted with chloroacetic acid in a mixture of acetic acid, acetic anhydride and fused sodium acetate to afford thiazolopyrimidines 10. The presence of the cyclized product as 10A is tentatively favored over the isomeric structure 10B was elucidated in light of its ^{1}H NMR, which showed that the pyrimidine proton of 10A is deshielded by about 0.7ppm relative to that of compound 9. The thiazolopyrimidines 10A were allowed to react with aromatic aldehyde in the presence of glacial acetic acid, acetic anhydride and fused sodium acetate to yield compound 11. The product 11 was obtained in better yields, in one pot reaction, by refluxing a mixture of 9, chloroacetic acid and aromatic aldehyde, fused sodium acetate and acetic anhydride in acetic acid.

Pharmacological Screening

Anticancer Activity: The results of the antitumor activity, the growth inhibition of 50% (GI_{50}) at a single concentration (10^{-4} M), for the synthesized compounds utilizing 55 different human tumor cell lines, representing cancers of the lung, colon, breast, ovary, renal, prostate and CNS in addition to leukemia and melanoma tumors are collected in Table 1.

The products showed to be active against non-small cell lung cancer with a remarkable activity against (NCI-H522) renal (ACHN) and leukemia (RPMI-8226) cell lines. The anticancer potency of the tested compounds is arranged in descending order as follow: 7a, 3g, 3a, 4a, 10, 9a, 11a, 8c, 8b.

Generally, we can conclude that the anticancer activities are due to the presence of nitrogen heterocyclic rings, the nitrile group (CN) and the pyridinethione moiety fused to nitrobenzoxepine ring.

Anti-inflammatory Activity

Purpose and Rational: For the determination of the antiphlogistic potency of the synthesized compounds, two standard tests were realized at 5 and 2.5 mg/kg rat body weight namely, the protection against Carrageenan® induced edema according to Winter *et al*., [22, 23] and the inhibition of plasma PGE2. The later is known as a good confirming indicator for the Carrageenan® induced rat paw edema. Regarding the protection against Carrageenan® induced edema, the tested ten compounds were found more potent than Diclofenac® as a reference drug (Table 2). Compounds 7a, 9c, 6d, 8c, 9d, 4c, 3g, 8b, 3f, 5c are arranged in descending order of anti-inflammatory potency.

Structure Activity Relationship (SAR): The pyridine nucleus showed to be essential for the anti-inflammatory activity and the cyanopyridinethione 7 is more potent than the pyrimidinethione 9 and the pyrimidineimine 8. In case of the pyrazole nucleus, it was found that its phenyl substitution in products 6 increase the potency meanwhile, N-acylation in products 5 decrease the activity in comparison with the unsubstituted ring of 4.

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