

**Anti-proliferative and pro-apoptotic activities of synthesized
3,4,5 tri-methoxy ciprofloxacin chalcone hybrid, through P₅₃ up-
regulation in HepG2 and MCF7 cell lines.**

"Anti-cancer activity of ciprofloxacin chalcone hybrid"

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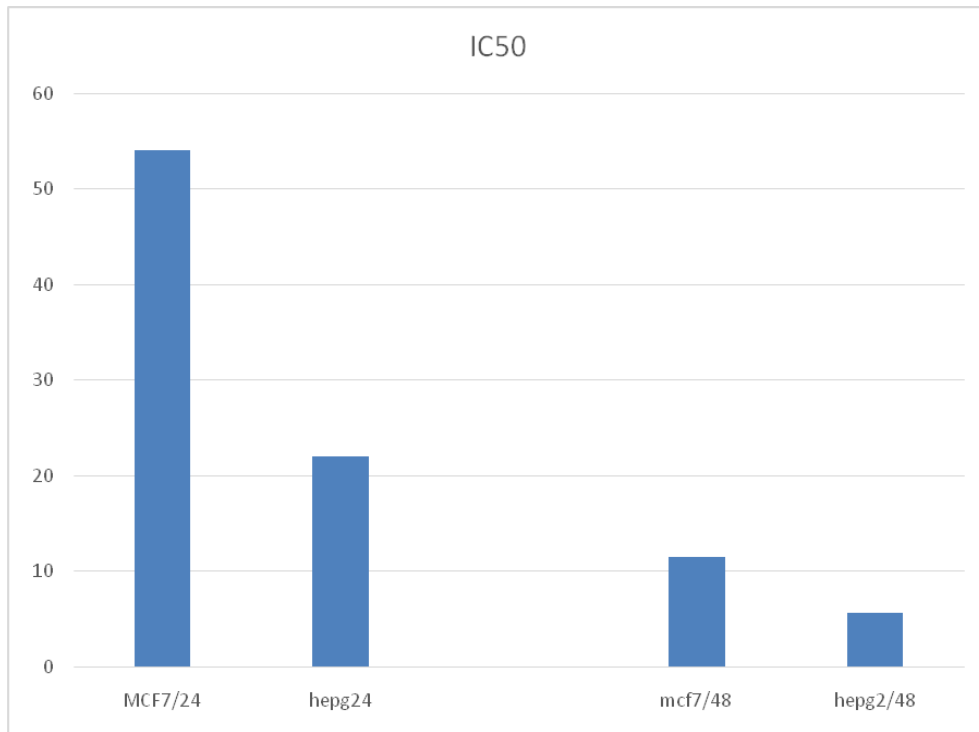
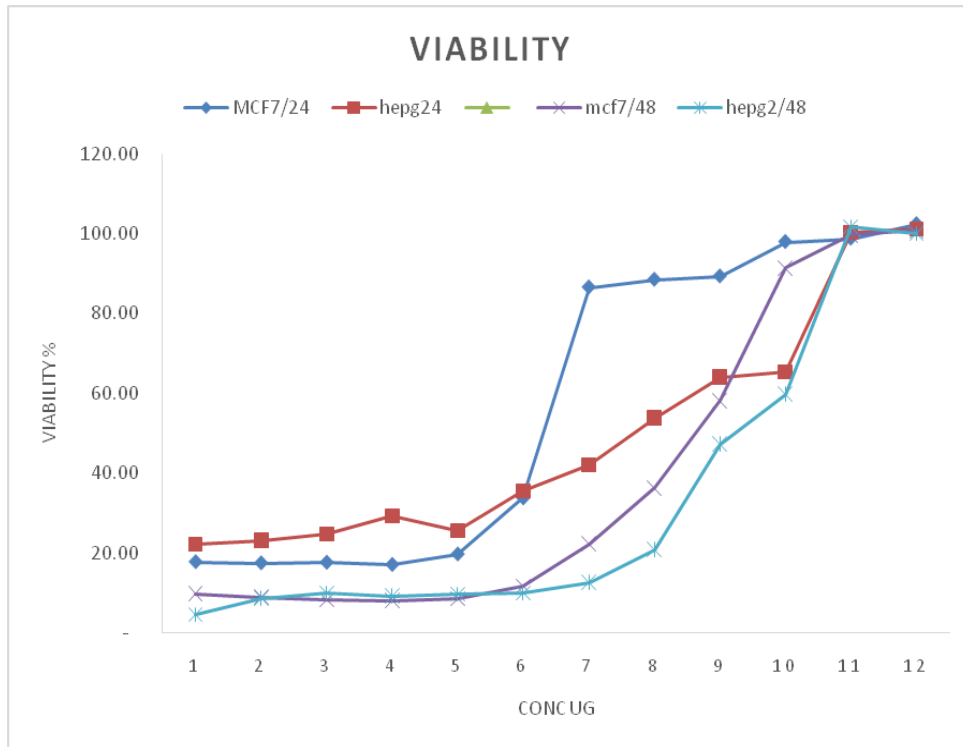
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S1- CELL VIABILITY TEST , IC₅₀ CALCULATION



| MCF7/24hrs | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| | 0.042 | 0.043 | 0.044 | 0.041 | 0.044 | 0.08 | 0.192 | 0.207 | 0.173 | 0.198 | 0.226 | 0.219 |
| | 0.044 | 0.042 | 0.042 | 0.037 | 0.043 | 0.075 | 0.174 | 0.272 | 0.214 | 0.26 | 0.252 | 0.274 |
| | 0.042 | 0.041 | 0.041 | 0.045 | 0.055 | 0.088 | 0.257 | 0.158 | 0.256 | 0.247 | 0.233 | 0.244 |
| | 0.042667 | 0.042 | 0.042333 | 0.041 | 0.047333 | 0.081 | 0.207667 | 0.212333 | 0.214333 | 0.235 | 0.237 | 0.245667 |
| | 17.77778 | 17.5 | 17.63889 | 17.08333 | 19.72222 | 33.75 | 86.52778 | 88.47222 | 89.30556 | 97.91667 | 98.75 | 102.3611 |
| | 0.251 | 0.252 | 0.254 | 0.23 | 0.221 | 0.218 | 0.268 | 0.271 | 0.222 | 0.227 | 0.225 | 0.278 |
| cc | | | | | | | | | | | | |
| HepG2 | 0.047 | 0.036 | 0.042 | 0.041 | 0.044 | 0.063 | 0.073 | 0.094 | 0.121 | 0.121 | 0.177 | 0.175 |
| | 0.039 | 0.04 | 0.046 | 0.069 | 0.044 | 0.065 | 0.07 | 0.084 | 0.115 | 0.11 | 0.18 | 0.169 |
| | 0.028 | 0.043 | 0.039 | 0.04 | 0.043 | 0.054 | 0.072 | 0.097 | 0.091 | 0.103 | 0.155 | 0.172 |
| | 0.038 | 0.039667 | 0.042333 | 0.05 | 0.043667 | 0.060667 | 0.071667 | 0.091667 | 0.109 | 0.111333 | 0.170667 | 0.172 |
| | 22.35294 | 23.33333 | 24.90196 | 29.41176 | 25.68627 | 35.68627 | 42.15686 | 53.92157 | 64.11765 | 65.4902 | 100.3922 | 101.1765 |
| cc | 0.143 | 0.196 | 0.138 | 0.172 | 0.16 | 0.171 | 0.156 | 0.125 | 0.198 | 0.145 | 0.207 | 0.197 |
| 48 hr | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | 0.081 | 0.048 | 0.046 | 0.045 | 0.051 | 0.073 | 0.116 | 0.265 | 0.436 | 0.595 | 0.522 | 0.522 |
| B | 0.054 | 0.049 | 0.045 | 0.043 | 0.044 | 0.05 | 0.103 | 0.154 | 0.187 | 0.531 | 0.547 | 0.547 |
| C | 0.026 | 0.049 | 0.045 | 0.044 | 0.045 | 0.067 | 0.144 | 0.17 | 0.319 | 0.354 | 0.544 | 0.563 |
| | 0.053667 | 0.048667 | 0.045333 | 0.044 | 0.046667 | 0.063333 | 0.121 | 0.196333 | 0.314 | 0.493333 | 0.537667 | 0.544 |
| | 9.938272 | 9.012346 | 8.395062 | 8.148148 | 8.641975 | 11.7284 | 22.40741 | 36.35802 | 58.14815 | 91.35802 | 99.5679 | 100.7407 |
| D | 0.488 | 0.521 | 0.743 | 0.667 | 0.46 | 0.687 | 0.4 | 0.412 | 0.734 | 0.486 | 0.564 | 0.415 |
| E | 0.0224 | 0.047 | 0.052 | 0.053 | 0.052 | 0.055 | 0.063 | 0.109 | 0.211 | 0.281 | 0.5 | 0.566 |
| F | 0.025 | 0.048 | 0.056 | 0.054 | 0.058 | 0.061 | 0.072 | 0.123 | 0.252 | 0.272 | 0.553 | 0.547 |
| G | 0.031 | 0.05 | 0.059 | 0.048 | 0.051 | 0.051 | 0.075 | 0.115 | 0.319 | 0.435 | 0.625 | 0.538 |
| | 0.026133 | 0.048333 | 0.055667 | 0.051667 | 0.053667 | 0.055667 | 0.07 | 0.115667 | 0.260667 | 0.329333 | 0.559333 | 0.550333 |
| | 4.751515 | 8.787879 | 10.12121 | 9.393939 | 9.757576 | 10.12121 | 12.72727 | 21.0303 | 47.39394 | 59.87879 | 101.697 | 100.0606 |
| H | 0.462 | 0.383 | 0.438 | 0.575 | 0.684 | 0.703 | 0.579 | 0.539 | 0.577 | 0.582 | 0.566 | 0.56 |

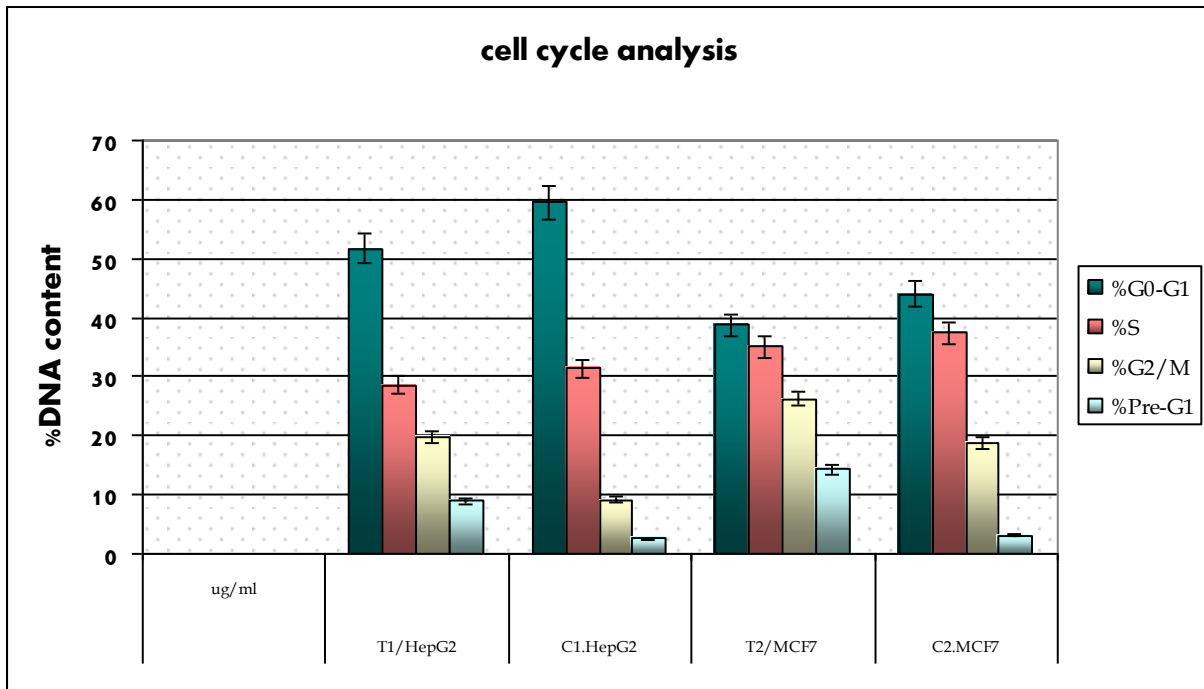
| | MCF7/24 | hepg24 | mcf7/48 | hepg2/48 |
|------|---------|--------|---------|----------|
| 2000 | 17.78 | 22.35 | 9.94 | 4.75 |
| 100 | 17.50 | 23.33 | 9.01 | 8.79 |
| 500 | 17.64 | 24.90 | 8.40 | 10.12 |
| 250 | 17.08 | 29.41 | 8.15 | 9.39 |
| 125 | 19.72 | 25.69 | 8.64 | 9.76 |
| 62.5 | 33.75 | 35.69 | 11.73 | 10.12 |
| 32 | 86.53 | 42.16 | 22.41 | 12.73 |
| 16 | 88.47 | 53.92 | 36.36 | 21.03 |

| | | | | |
|---|--------|--------|--------|--------|
| 8 | 89.31 | 64.12 | 58.15 | 47.39 |
| 4 | 97.92 | 65.49 | 91.36 | 59.88 |
| 2 | 98.75 | 100.39 | 99.57 | 101.70 |
| 1 | 102.36 | 101.18 | 100.74 | 100.06 |

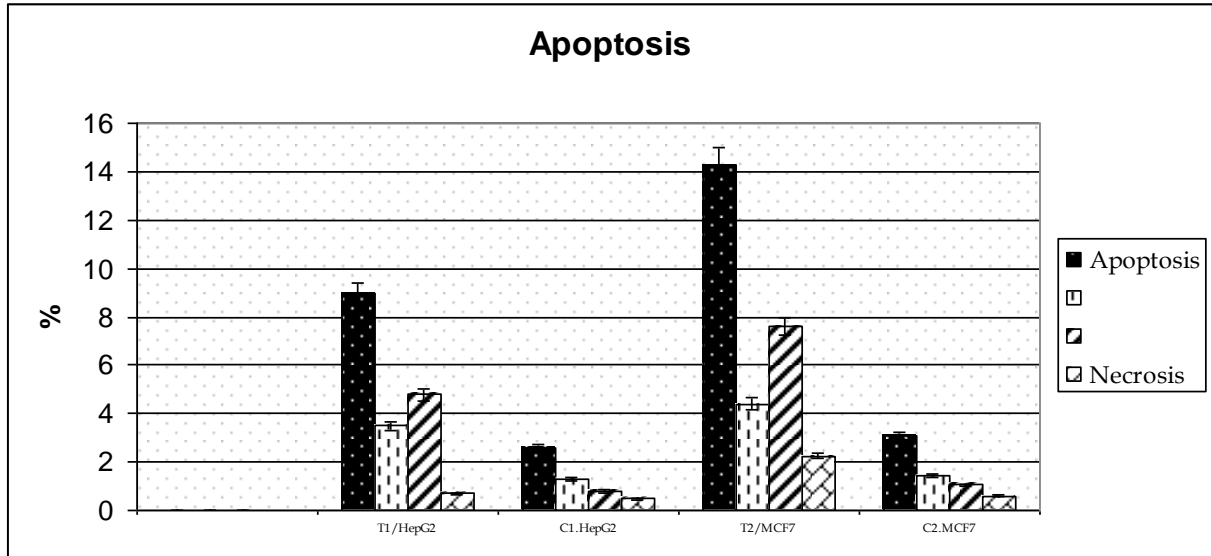
| | | | | |
|------|---------|--------|---------|----------|
| | MCF7/24 | hepg24 | mcf7/48 | hepg2/48 |
| IC50 | 54 | 22 | 11.5 | 5.6 |

S2- CELL CYCLE ANALYSIS

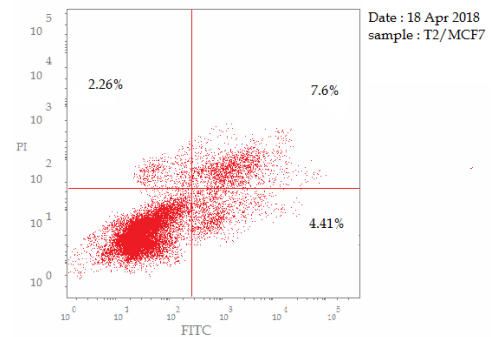
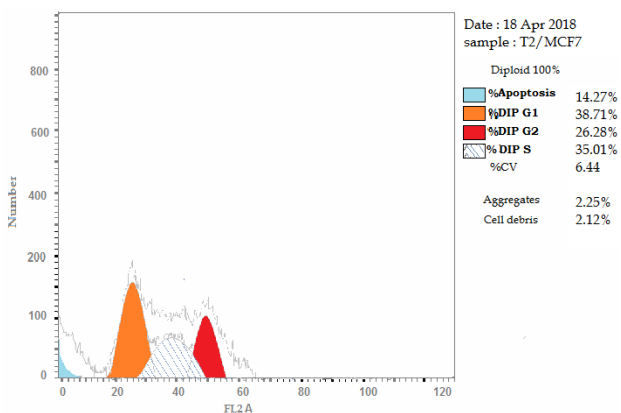
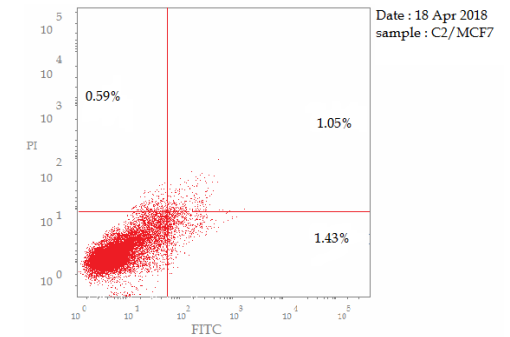
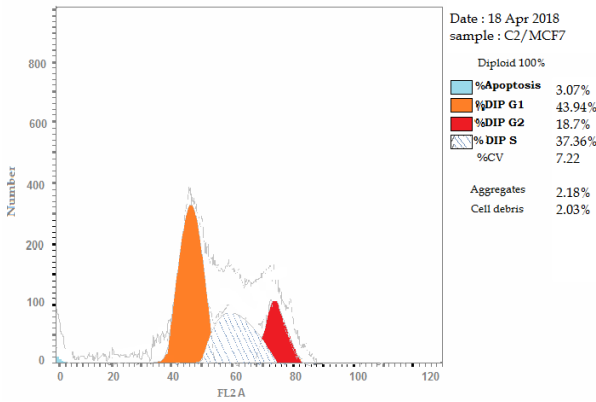
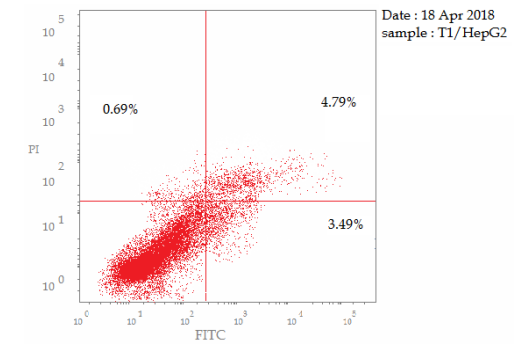
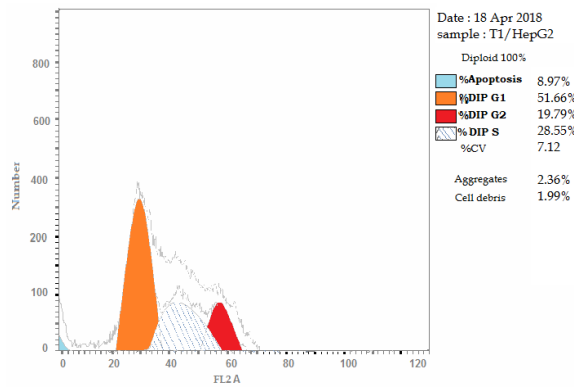
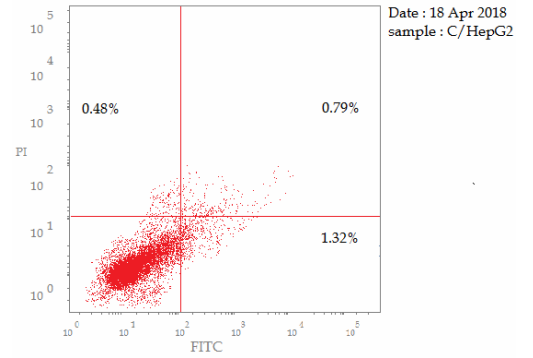
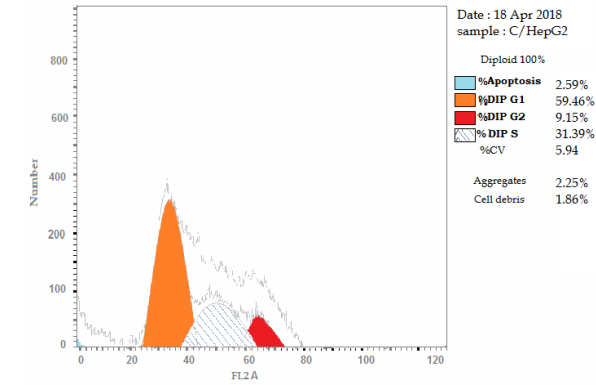
| Sample data | | | Results | | | | |
|-------------|-------------|------------------|---------|-------|-------|---------|--|
| Ser | Sample code | Used conc. ug/ml | %G0-G1 | %S | %G2/M | %Pre-G1 | Comment |
| 1 | T1/HepG2 | | 51.66 | 28.55 | 19.79 | 8.97 | PreG1apoptosis&Cell growth arrest@G2/M |
| 2 | C1.HepG2 | | 59.46 | 31.39 | 9.15 | 2.59 | PreG1apoptosis&Cell growth arrest@G2/M |
| 3 | T2/MCF7 | | 38.71 | 35.01 | 26.28 | 14.27 | PreG1apoptosis&Cell growth arrest@G2/M |
| 4 | C2.MCF7 | | 43.94 | 37.36 | 18.7 | 3.07 | |



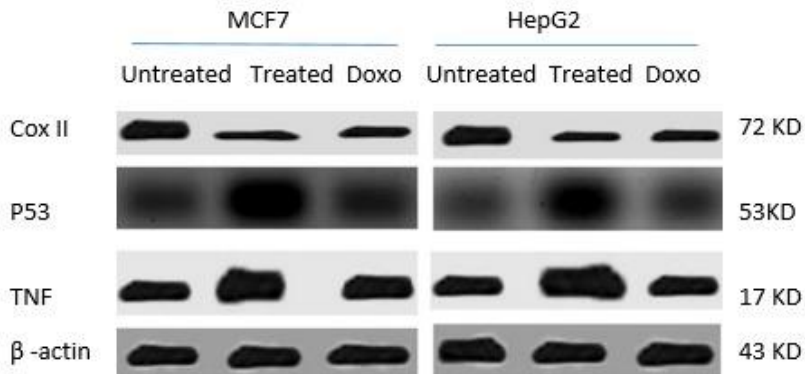
| | conc. ug/ml | Apoptosis | | | Necrosis |
|---|----------------|-----------|-------|------|----------|
| | | Total | Early | Late | |
| 1 | T1/HepG2 | 8.97 | 3.49 | 4.79 | 0.69 |
| 2 | C1.HepG2 | 2.59 | 1.32 | 0.79 | 0.48 |
| 3 | T2/MCF7 | 14.27 | 4.41 | 7.6 | 2.26 |
| 4 | C2.MCF7 | 3.07 | 1.43 | 1.05 | 0.59 |



Detailed results



S3- WESTERN BLOTTING



p53

| | MCF7 | | | HepG2 | | |
|---------|---------|-------------|----------|---------|-------------|----------|
| | Control | Tested drug | doxo | Control | Tested drug | doxo |
| trial 1 | 1 | 2.5 | 1.75 | 1 | 3 | 1.9 |
| trial 2 | 1 | 2.51 | 1.751 | 1 | 3 | 1.99 |
| trial 3 | 1 | 2.499 | 1.749 | 1 | 3.1 | 1.91 |
| trial 4 | 1 | 2.5 | 1.75 | 1 | 3.1 | 1.9 |
| trial 5 | 1 | 2.5 | 1.75 | 1 | 3 | 1.9 |
| avg | 1 | 2.5018 | 1.75 | 1 | 3.04 | 1.92 |
| st dev | 0 | 0.004118 | 0.000632 | 0 | 0.04899 | 0.035214 |

cox2

| | MCF7 | | | HepG2 | | |
|---------|---------|-------------|----------|---------|-------------|----------|
| | Control | Tested drug | doxo | Control | Tested drug | doxo |
| trial 1 | 1 | 0.293097 | 0.4 | 1 | 0.31 | 0.5 |
| trial 2 | 1 | 0.293098 | 0.41 | 1 | 0.32 | 0.49 |
| trial 3 | 1 | 0.291098 | 0.39 | 1 | 0.3 | 0.5 |
| trial 4 | 1 | 0.293098 | 0.4 | 1 | 0.31 | 0.48 |
| trial 5 | 1 | 0.292098 | 0.4 | 1 | 0.31 | 0.49 |
| avg | 1 | 0.292498 | 0.4 | 1 | 0.31 | 0.492 |
| st dev | 0 | 0.0008 | 0.006325 | 0 | 0.006325 | 0.007483 |

| | TNF-alpha | | | | | |
|---------|-----------|-------------|----------|---------|-------------|-------|
| | MCF7 | | | HepG2 | | |
| | Control | Tested drug | doxo | Control | Tested drug | doxo |
| trial 1 | 1 | 1.87706 | 1.8 | 1 | 2.5 | 1.35 |
| trial 2 | 1 | 1.87806 | 1.81 | 1 | 2.51 | 1.34 |
| trial 3 | 1 | 1.87906 | 1.79 | 1 | 2.52 | 1.35 |
| trial 4 | 1 | 1.87806 | 1.8 | 1 | 2.5 | 1.33 |
| trial 5 | 1 | 1.87806 | 1.8 | 1 | 2.5 | 1.35 |
| avg | 1 | 1.87806 | 1.8 | 1 | 2.506 | 1.344 |
| st dev | 0 | 0.000632 | 0.006325 | 0 | 0.008 | 0.008 |

S4- RT-PCR RESULTS

| | p53 | | | | | |
|---------|---------|-------------|----------|---------|-------------|----------|
| | MCF7 | | | HepG2 | | |
| | Control | Tested drug | doxo | Control | Tested drug | doxo |
| trial 1 | 1 | 15.349 | 6 | 1 | 6.58 | 3.7 |
| trial 2 | 1 | 15.34 | 6.1 | 1 | 6.58 | 3.6 |
| trial 3 | 1 | 15.348 | 5.9 | 1 | 6.59 | 3.8 |
| trial 4 | 1 | 15.349 | 6 | 1 | 6.57 | 3.71 |
| trial 5 | 1 | 15.348 | 6 | 1 | 6.58 | 3.69 |
| avg | 1 | 15.3468 | 6 | 1 | 6.58 | 3.7 |
| st dev | 0 | 0.003429 | 0.063246 | 0 | 0.006325 | 0.063561 |

| | cox2 | | | | | |
|---------|---------|-------------|----------|---------|-------------|----------|
| | MCF7 | | | HepG2 | | |
| | Control | Tested drug | doxo | Control | Tested drug | doxo |
| trial 1 | 1 | 0.393097 | 0.5 | 1 | 0.41 | 0.6 |
| trial 2 | 1 | 0.393098 | 0.51 | 1 | 0.42 | 0.59 |
| trial 3 | 1 | 0.391098 | 0.49 | 1 | 0.4 | 0.6 |
| trial 4 | 1 | 0.393098 | 0.5 | 1 | 0.41 | 0.58 |
| trial 5 | 1 | 0.392098 | 0.5 | 1 | 0.41 | 0.59 |
| avg | 1 | 0.392498 | 0.5 | 1 | 0.41 | 0.592 |
| st dev | 0 | 0.0008 | 0.006325 | 0 | 0.006324555 | 0.007483 |

| | TNF-alpha | | | | | |
|---------|-----------|-------------|----------|---------|-------------|-------|
| | MCF7 | | | HepG2 | | |
| | Control | Tested drug | doxo | Control | Tested drug | doxo |
| trial 1 | 1 | 2.87706 | 2.2 | 1 | 3.5 | 1.75 |
| trial 2 | 1 | 2.87806 | 2.21 | 1 | 3.51 | 1.74 |
| trial 3 | 1 | 2.87906 | 2.19 | 1 | 3.52 | 1.75 |
| trial 4 | 1 | 2.87806 | 2.2 | 1 | 3.5 | 1.73 |
| trial 5 | 1 | 2.87806 | 2.2 | 1 | 3.5 | 1.75 |
| avg | 1 | 2.87806 | 2.2 | 1 | 3.506 | 1.744 |
| st dev | 0 | 0.000632 | 0.006325 | 0 | 0.008 | 0.008 |

primers



Quality
Endorsed
Company
ISO 9001 Lic 21313
SAI Global

p53 F 5'- CCCCTCCTGGCCCCTGTCATCTTC-3',
p53 R 5'-GCAGCGCCTCACAACTCCGTCAT-3'.

TNF- α F 5'- TTGACCTCAGCGCTGAGTTG -3'
TNF- α R 5'- CCTGTAGCCCACGTCGTAGC -3'

COX-2 F 5'- ACTGTACGGGGTTTGTGACTAG -3'
COX-2 F 5'- ACTGTACGGGGTTTGTGACTAG -3'

β -actin F 5'-GTGACATCCACACCCAGAGG-3'
 β -actin R 5'-ACAGGATGTCAAACCTGCCC-3'